Entomopathogenic fungi in New Zealand native forests: the genera *Beauveria* and *Isaria*

A thesis submitted in partial fulfilment of the requirements for the Degree of

Doctor of Philosophy

at the University of Canterbury

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2009

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ABSTRACT

Species of the entomopathogenic fungal genera *Beauveria* and *Isaria* were collected and isolated from diverse arthropod hosts in native forests. Morphological observations and analysis of DNA sequence data from three nuclear gene regions were used to identify taxa and examine phylogenetic relationships. Several new host associations were found for *Beauveria* species. The recently described species *Beauveria malawiensis* is reported infecting insects in New Zealand for the first time. The known host range of this species is extended to include Hemiptera, Hymenoptera, Orthoptera and Phasmatodea. *Beauveria caledonica,* previously only recorded in New Zealand from introduced bark beetles in pine forests, is reported for the first time in native forests and on non-coleopteran hosts. Insect bioassays of *Beauveria bassiana* and *B. malawiensis* isolates were conducted to examine host specificity and identify strains with potential for controlling introduced *Vespula* wasps. Bioassay results generally suggested that strains did not have specific host requirements and isolates of both species were found to be pathogenic towards *Vespula* larvae. Morphological and molecular data indicated that two distinct groups of *Isaria farinosa*-like fungi occur in New Zealand and should be recognised as separate species. Similarly, two discrete groups resembling *Isaria cicadae* were identified. The results indicate that species diversity in *Isaria* has been previously underestimated in New Zealand.

CHAPTER ONE: INTRODUCTION

The ability to infect insects and other arthropods has arisen independently in all of the traditionally recognised fungal phyla (Humber 2008; Rehner 2009). Entomopathogenic fungi are considered to play an important role in the natural regulation of arthropod populations (Evans 1982). While substantial research has been directed towards the application of these fungi as biological control agents, this has mainly focused on a few species which are commonly associated with agricultural pests (Hajek & St Leger 1994; Hywel Jones 2002). Most records of entomopathogenic fungi in New Zealand have been from agricultural or exotic forest habitats, and currently little is known about the taxonomic diversity and ecology of these fungi in native forests.

1.1 Historical perspective

The earliest accounts of insect fungi are found for species with traditional ethnomycological uses. The lepidopteran pathogen *Ophiocordyceps sinensis* is particularly prized in Chinese medicine and may have been known and used for at least 2000 years (Lloyd 1919). Another medicinal species, *Cordyceps sobolifera,* was first recorded in Chinese literature as early as 300 AD (Wang 1987). Silkworms infected with *Beauveria bassiana* were also valued for their medicinal properties in China, Japan, and Korea, with records dating back to 900 AD (Kikuchi *et al.* 2004; Pemberton 1999; Steinhaus 1956, 1975). In New Zealand, *Ophiocordyceps robertsii* was traditionally used by Maori, mainly as a tattoo pigment, but also as a food and medicine (Fuller *et al.* 2004; Riley 1994).

Reports of 'vegetating' insects first appeared in Western literature in the eighteenth century, although it was not initially recognised that this phenomenon was caused by fungi. Specimens of *Ophiocordyceps sinensis* sent to France and examined by Reamur in 1726 were first interpreted as larvae which had become attached to plant roots (Cooke 1892). The descriptions by Torrubia in 1754 of 'trees' growing from dead wasps and the 'vegetable fly' reported by Edwards to the Royal Society in 1761 caused much debate in scientific circles. Both were considered at first to be examples of transmutation, *i.e.* insects that changed into plants (Fraser 1994; Ramsbottom 1941). Examination of the vegetable fly later showed that this was actually a fungus growing from dead cicada nymphs which was named as *Clavaria (=Cordyceps) sobolifera* (Hill in Watson 1764). Edwards (1764) suggested that the wasps observed by Torrubia were associated with a similar fungus. The fungal nature of both *C. sobolifera* and *O. sinensis* was later confirmed by de Bonderoy in 1769 (Cooke 1892).

Following these early observations, fungi continued to be described from insects and were generally considered to be saprotrophic, developing only on dead hosts (Ramsbottom 1941). The idea that these fungi possibly developed on living insects and caused the death of their hosts was suggested by several authors (Cist 1824; Kirby 1826; Mitchill 1827) but not proved experimentally until the pioneering work of the Italian Agostino Bassi. In 1807 Bassi began an extensive series of experiments to determine the nature of the 'mark' or 'muscardine' disease which had become a serious problem in the silk industries of Italy and France. A prevailing assumption of the time was that environmental conditions during silkworm breeding caused the spontaneous development of the disease (Steinhaus 1956, 1975). Bassi determined that the disease did not develop spontaneously and was caused by a fungus. Bassi's findings, published in 1835, showed for the first time that fungi could cause disease. Balsamo-Crivelli provided a taxonomic description of the fungus which was named *Botrytis (=Beauveria) bassiana* in honour of Bassi's achievements (Major 1944; Steinhaus 1956, 1975).

1.2 Development of taxonomy

During the latter part of the nineteenth century, increasing numbers of entomopathogenic fungi from around the world were examined by European and American mycologists (Samson *et al.* 1988). Early reviews by Gray (1858) and Cooke (1892) show the development of knowledge concerning entomopathogenic fungi during this period. Excluding the parasitic Laboulbeniales, Cooke (1892) listed over 100 entomopathogenic species and placed these in three main groups: *Cordyceps* and *Isaria*, Entomophthorales, and miscellaneous 'moulds'. Included were two species described from New Zealand material: *Cordyceps robertsii* (=*Ophiocordyceps robertsii*) (Hooker 1837) and *Cordyceps sinclairii* (=*Isaria cicadae*) (Berkeley 1855). Berkeley (1855) had also described the entomopathogenic species *Aschersonia duplex* from a New Zealand specimen, but its role as an insect pathogen was not recognised at this time.

A major development in nineteenth century mycology was the discovery that many fungi are pleomorphic and have two reproductive states. It was recognized that the sexual fruiting body (now known as the teleomorph) of ascomycete species may also be associated with an asexual conidial state (anamorph), and that these two stages often developed independently (Reynolds 1993; Seifert & Gams 2001). Most anamorphic fungi described from insects in the nineteenth century were placed in the genus *Isaria* and although often without direct evidence, it became widely accepted that these were conidial forms of *Cordyceps s*pecies (*e.g.* Cooke 1892; Massee 1895).

Significant taxonomic advances were made in the first half of the twentieth century, with more emphasis placed upon microscopic characters, which were often neglected in earlier descriptions. Petch described over 74 entomopathogenic species from Sri Lanka between 1931 and 1944, many of which are still valid (Hywel-Jones 1997a; Samson *et al.* 1988). Major contributions to the taxonomy of *Cordyceps* and allied species were also made by Kobayasi in Japan and Mains in North America (*e.g.* Kobayasi 1939, 1941; Mains 1940, 1947, 1949, 1950). During this period several new *Cordyceps* species were described from New Zealand material by Cunningham (1921, 1922) and Lloyd (1915, 1920). As part of a comprehensive study of the Hypocreales in New Zealand, Dingley (1951, 1953, 1954) examined many entomopathogenic fungi and described several new species, mainly from scale insects.

Anamorphic fungi were initially classified according to the system proposed by Saccardo in the late nineteenth century. The Saccardoan classification system separated genera based on morphological characters such as general conidiomatal form and also pigmentation, colour, shape, and septation of conidia (Seifert & Gams 2001; Sutton 1996). Later authors (notably Vuillemin in 1910-12) began to place more taxonomic emphasis on the morphology of conidiogenous cells and the method of conidium production (conidiogenesis) (Humber 2000; Sutton 1996). Hughes (1953) integrated these earlier ideas into a revised classification system for anamorphic fungi in which the mode of conidiogenesis was the primary taxonomic character used to define genera.

Taxonomic studies of entomopathogenic fungi greatly increased from the nineteen-seventies. Revised generic concepts following Hughes (1953) are reflected in monographs on important anamorphic genera including *Beauveria* (de Hoog 1972), *Metarhizium* (Tulloch 1976), and *Paecilomyces* (Samson 1974). Evans (1974) made extensive collections of fungi infecting insects and spiders in Ghana and noted that many species were difficult to identify using existing literature due to few previous collections, lack of type specimens and inadequate descriptions. A series of subsequent papers examined species in poorly known anamorphic genera including *Akanthomyces* (Samson & Evans 1974), *Gibellula* (Samson & Evans 1973), *Hymenostilbe* (Samson & Evans 1975), and *Nomuraea* (Samson & Evans 1977). Evans later collected in South America and his collections from the tropics included many new species and provided much insight into the ecology of entomopathogenic fungi in tropical forests (*e.g.* Evans 1974, 1982; Evans & Samson 1982a, 1982b, 1984). Kobayasi and Shimizu published extensively on *Cordyceps* and *Torrubiella* between 1976 and 1982, describing species which had been collected over the preceding 30-40 years, mainly from Japan. These authors made substantial taxonomic

contributions to both genera during this period, describing 84 new species in *Cordyceps* and 27 in *Torrubiella* (Kobayasi 1982; Kobayasi & Shimizu 1982).

More recently, the recognition of entomopathogenic fungi as an importance source of novel metabolites with pharmaceutical applications (see Isaka *et al.* 2005) has led to additional interest in isolating these fungi from the tropics. A long-running study has demonstrated significant biodiversity of entomopathogenic fungi in tropical forest in Thailand (see Hywel-Jones 2001). An important aspect of this research has been a focus on obtaining cultures and determining anamorph-teleomorph connections (*e.g.* Hywel-Jones 1995a, 1995b, 1996, 1997b; Hywel-Jones & Sivichai 1995), which were both often neglected by earlier workers.

Few studies have examined the taxonomy of entomopathogenic fungi in New Zealand. Following the publications of Dingley (1953, 1954) only two new entomopathogenic species have been described from this country: *Coelomomyces opifexi* from the mosquito *Opifex fuscus* (Pillai & Smith 1968), and *Tolypocladium extinguens* from the glow-worm *Arachnocampa luminosa* (Samson & Soares 1984). While over 70 entomopathogenic fungal species have been recorded in New Zealand (Pennycook & Galloway 2004), in most cases these have also been recorded from other countries. Buchanan *et al.* (2004) suggested that many entomopathogenic species recorded in New Zealand may represent new species that have been misidentified as morphologically similar taxa.

1.3 Impact of molecular techniques on taxonomy

The traditional approach to fungal taxonomy based on morphological characters has often been problematic in entomopathogenic fungi, especially in anamorphic species (Humber 2000; Inglis & Tigano-Milani 2006; Oborník *et al.* 2001; Samson 1995). While most anamorphic genera are easily distinguished through their characteristic modes of conidiogenesis, only a limited range of morphological characters are used to separate species. These characters often display considerable morphological plasticity in the environment or in artificial culture. For example, in *Beauveria* and *Metarhizium* conidial shape and size are the only reliable morphological characters for species identification (Glare *et al.* 1996a, 1996b; Mugnai *et al.* 1989; Rehner 2005; Rehner & Buckley 2005). However, in both genera spore dimensions demonstrate a high degree of intraspecific variability, especially in culture, and isolates may show characteristics which are intermediate between two different species (Glare *et al.* 1996, 1996b; Glare & Inwood 1998; Mugnai *et al.* 1989).

Another problem is that morphological characters do not necessarily reflect phylogenetic relationships. The relatively simple mechanisms associated with conidial production may lead to similar morphologies and modes of development occurring in unrelated groups through convergent evolution (Humber 2000). Classification of *Verticillium* based on morphology (Gams 1971) resulted in an unnatural grouping which included insect-pathogenic and plant-pathogenic species, with corresponding teleomorphs in two unrelated ascomycete families. A parallel situation arose in the classification of *Paecilomyces* by Samson (1974), with the inclusion of both entomopathogenic and thermophilic species in the genus, again with each group having unrelated teleomorphs.

Modern molecular techniques allow the application of more objective criteria for fungal identification and classification, with DNA sequences providing large numbers of taxonomically informative characters (Taylor 1993). Sequence data has become an important tool for differentiating species, determining anamorph-teleomorph connections and inferring phylogenetic relationships. Recent molecular phylogenetic studies have led to a major higher-level reclassification of fungi (Hibbet *et al.* 2007; James *et al.* 2006) and significant taxonomic revisions in entomopathogenic genera (*e.g.* Chaverri *et al.* 2008; Johnson *et al.* 2009; Luangsa-ard *et al.* 2005; Sung *et al.* 2007a; Zare & Gams 2001).

Nuclear ribosomal DNA (rDNA) has been the most commonly sequenced region for fungal identification and systematics (Bruns & Shefferson 2004; Geiser 2004; Lutzoni *et al.* 2004). The rDNA repeat unit includes three genes encoding for the small subunit (SSU or 18S), 5.8S subunit, and large subunit (28S or LSU) of ribosomal RNA. Each gene is separated by non-coding internal transcribed spacer (ITS) regions and the whole unit is repeated in hundreds of copies along the genome, with each copy separated by the non-coding intergenic spacer (IGS) region. The ribosomal genes and spacer regions evolve at different rates so can be informative at different taxonomic levels (Bruns *et al.* 1991). The 18S and 28S genes are highly conserved and have been mainly used to examine broad phylogenetic relationships among fungi *i.e.* at or above generic level. The ITS region generally shows variation at around species level and has been used extensively for species identification and phylogenetic analyses within genera (Lutzoni *et al.* 2004; Geiser 2004; Bridge *et al.* 2005). However, the rate of divergence in ITS sequences may vary between different fungal groups. Some species demonstrate a high degree of intraspecific ITS variability (Seifert *et al.* 1995; Nilsson *et al.* 2008), while in certain genera very closely related species show little sequence difference (Bruns 2001; Lieckfeldt & Seifert 2000). Despite these limitations, ITS sequences for a wide range of fungal species are available in

public databases and the region is likely to become the standard 'barcoding' locus for fungal identification (Seifert & Crous 2008).

Sequences from protein-coding genes typically provide greater taxonomic resolution and have been increasingly used to complement or replace ribosomal DNA sequences in phylogenetic analyses. Protein coding loci which have been commonly used for fungal systematics include translation elongation factor 1-α, β-tubulin, ribosomal polymerase B, and mitochondrial ATPase6 (Bruns & Shefferson 2004; Lutzoni *et al.* 2004). The higher resolution associated with these genes is mainly due to the presence of non-coding intron regions which may provide phylogenetic signals 3-6 times stronger than ITS sequences (Geiser 2004). Intron regions of protein-coding genes have been particularly useful for species identification in genera such as *Fusarium*, where ITS sequences cannot reliably separate all species (Geiser *et al.* 2004; O'Donnell & Cigelnik 1997). The exon (coding) regions are more informative at higher taxonomic levels and may be used to clarify relationships which can not be completely resolved with ribosomal gene sequences (Geiser 2004; Lutzoni *et al.* 2004). A particular advantage of these loci over ribosomal genes is that sequences from more distantly related taxa are much easier to align (Bruns 2001; Bruns & Shefferson 2004). Recent phylogenetic studies have used a multi-locus approach, combining sequences from several protein-coding and ribosomal genes to produce robust, highly resolved phylogenies that more accurately reflect evolutionary relationships (*e.g.* Hibbet *et al.* 2007; James *et al.* 2006; Lutzoni *et al.* 2004; Matheny *et al.* 2007; Spatafora *et al.* 2007; Sung *et al.* 2007a, 2007b).

Molecular phylogenetic analyses have established comprehensive sequence datasets providing a common framework for identification of entomopathogenic fungi. Importantly this data is derived from specimens that have been well-characterised morphologically, in many cases from type specimens. Sequence data has been increasingly used for routine identification of New Zealand isolates, although currently this approach has only been applied to a limited number of species (Glare 2004; Glare *et al.* 2008; Marshall *et al.* 2003; Reay *et al.* 2007, 2008).

1.4 Host specificity, infection, and dispersal

Species of entomopathogenic fungi show considerable variation in host specificity and include both fastidious pathogens with restricted host ranges and opportunistic, broad host- range pathogens (Fargues & Remaudière 1977). Adaptation towards specific host groups and their habitats is reflected in the morphological diversity encountered amongst entomopathogenic taxa. Entomophthoralean species are generally characterised by narrow host ranges and mainly infect foliar insects and mites (Evans 1989; Pell *et al.* 2001). The most extreme examples of host-

specificity in this group are found in the genus *Massospora*, where each species only infects a single genus of cicada (Soper 1974). Within the Hypocreales, most species of *Cordyceps*, *Ophiocordyceps* and *Torrubiella* are restricted to a single arthropod family or order (Kobayasi & Shimizu 1982; Sung *et al.* 2007a). These fungi are usually further specialised towards a particular developmental stage of the host *e.g.* larvae or pupae. Species in *Hypocrella*, *Moelleriella* and *Samuelsia* are only found as pathogens of scale insects and whiteflies (Chaverri *et al.* 2008), while *Orbiocrella, Conoideocrella and Regiocrella* species are restricted to scale insects (Chaverri *et al.* 2005; Johnson *et al.* 2009). Some taxa only infect particular groups of non-insect arthropods, *e.g. Gibellula* species are specific pathogens of hunting spiders (Hywel-Jones 2001), while several species of *Hirsutella* are limited to acarine hosts (Minter *et al.* 1983; Samson *et al.* 1980). In contrast, species such as *Beauveria bassiana* and *Metarhizium anisopliae* display wide host ranges. *Beauveria bassiana* has been recorded from over 700 host species in 15 insect orders and is also known to infect mites (Acari) (Li 1988). Host records of *M. anisopliae* include over 200 species in 11 insect orders (Zimmermann 2007b). However, individual isolates may have more restricted host preferences and it is generally accepted that these species include both host-specific and generalist strains (Bidochka & Small 2005; Goettel *et al.* 1990; Vestergaard *et al.* 2003).

In most cases, fungal pathogens gain access to nutrients in the arthropod haemocoel by direct penetration of the host cuticle (Payne *et al.* 1988; St. Leger 1991). While the exact mechanisms of host specificity remain unclear, the ability of host specific pathogens to cause disease when injected directly into the haemocoel of non-host insects suggests that specificity is regulated at the cuticular level (Goettel *et al.* 1990). When spores come into contact with a susceptible host the following series of events is initiated: (1) spore attachment; (2) germination and production of germ tubes or appressoria; and (3) cuticle penetration using enzymes and mechanical pressure. Each of these stages may be mediated by fungal recognition of physical or chemical cues from the host cuticle (St Leger 1993).

Spores must remain in contact with the host cuticle for a sufficient length of time to allow subsequent germination and penetration (St Leger 1991). For many fungal species initial spore attachment is thought to be passive and nonspecific. In fungi with dry, hydrophobic conidia *e.g. Beauveria*, *Metarhizium,* and *Nomuraea*, attachment to host and non-host insects is mediated by hydrophobic interactions between conidia and the waxy, hydrophobic insect cuticle. Although this mechanism is not specific for particular hosts it allows for preferential binding of conidia to insect cuticle rather than other substrates (Boucias *et al.* 1988). Proteins and carbohydrates may also be involved in the initial adhesion process as conidia of *Beauveria bassiana* treated with

proteases and glycosidases showed reduced attachment to hydrophobic substrates (Holder & Keyani 2005). Hydrophilic conidia with a mucus coating are produced by several genera including *Aschersonia, Hirsutella, Lecanicillium*, and some members of the Entomophthorales. The sticky, mucus coat is likely to be involved in the passive attachment of conidia to insect cuticle (Boucias & Pendland 1991; Hajek 1997).

Selective attachment has also been demonstrated in host-specific isolates of some species. Vey *et al.* (1982) found that a host-specific strain of *Metarhizium anisopliae* that infected *Cetonia auruta* (Coleoptera) larvae attached poorly to non-host larvae. Similarly, aphid-pathogenic strains of *Verticillium lecanii* did not adhere to non-host insects (Sitch & Jackson 1997). Specific attachment of fungal pathogens is generally thought to be determined through binding of complementary molecules on the surfaces of the host and the pathogen (Manocha & Chen 1990). In entomopathogenic fungi this process may involve recognition of carbohydrates on the cuticle by carbohydrate-binding proteins on the surface of the fungal spore (Boucias & Pendland 1991; Kerwin & Washino 1986). Once attached, spores may require nutrients from the host surface to initiate germination and subsequent development. A variety of potential carbon and nitrogen sources are present on arthropod cuticles including carbohydrates, amino acids, peptides, fatty acids and lipids (Jarrold *et al.* 2007). Fungi with broad host ranges are thought to have relatively non-specific nutritional requirements for germination. Conidia of *Beauveria bassiana* were found to germinate and develop in response to a wide range of carbon and nitrogen sources (Smith & Grula 1981), including glucosamines and amino acids present on host cuticles (Woods & Grula 1984). Pathogens with restricted host ranges may have more specific requirements for germination. In the lepidopteran pathogen *Nomuraea rileyi,* conidial germination was specifically induced by lipids extracted from host cuticles (Boucias & Pendland 1984). St Leger *et al.* (1992b, 1994) showed that host-specific strains of *M. anisopliae* differed in their ability to germinate under various nutrient conditions and that these differences were frequently host-related.

Entomopathogenic fungi use a combination of enzymatic degradation and mechanical pressure to penetrate arthropod cuticle (St Leger 1995), which is a complex composite of proteins, lipids and chitin (Andersen *et al.* 1995). Because protein may constitute up to 70% of the cuticle (Andersen *et al.* 1995), proteases are of major importance in the penetration process. Proteases have been studied in most detail in *M. anisopliae* which produces multiple isoforms of several cuticle-degrading proteases including subtilisins (Pr1), trypsins (Pr2), chymotrypsins, and metalloproteases. Subtilisin-like proteases are also produced by other entomopathogenic fungi including species in *Aschersonia, Beauveria, Isaria, Lecanicillium* and *Nomuraea* (Castellanos-

Moguel *et al.* 2007; Charnley 2003). The types of proteins present in cuticle vary according to arthropod species and developmental stage (Andersen *et al.* 1995; Norup *et al.* 1996). Host range may therefore depend on the ability of a fungal isolate to produce the appropriate enzymes for degradation of specific host proteins (Bye & Charnley 2008). Freimoser *et al.* (2005) found that protease genes in *M. anisopliae* were differentially expressed in response to different insect cuticles and suggested that broad host range may be correlated with the ability to regulate the production of a variety of specific proteases.

Following penetration, fungi proliferate vegetatively within the haemocoel of the host as yeast-like blastospores, hyphal bodies, or wall-less protoplasts. These growth forms provide increased surface area for nutrient acquisition, and allow rapid circulation within the haemocoel which aids in colonisation and may help dissipate the immune responses of the host (Clarkson & Charnley 1996; Hajek 1997). Other mechanisms implicated in evasion of host defence responses include non-recognition of surface components of fungal cells by host haemocytes and the production of secondary metabolites which suppress the host defence system (Gillespie *et al.* 2000; Samson *et al.* 1988). Entomopathogenic fungi employ two basic nutritional strategies during colonisation of their hosts. Entomophthoralean species are characteristically biotrophic and host death is caused by depletion of available nutrients in the haemocoel. Fungal growth ceases when sporulation occurs soon after host death, or in some cases (*e.g.* in *Massospora* or *Strongwellsea* species) while the host is still living (Evans 1988; Pell *et al.* 2001). In contrast, hypocrealean species are hemibiotrophic and following an initial biotrophic phase, the pathogen produces toxic secondary metabolites causing host death, after which the fungus lives saprotrophically and sporulates on the dead host (Roy *et al.* 2006).

Entomopathogenic fungi have evolved a range of mechanisms for spore dispersal which are adapted to particular hosts and their habitats. Entomophthoralean species typically infect exposed, foliar hosts and produce short-lived primary conidia which are actively discharged from simple conidiophores on the host cadaver. Generally, primary conidia that land on non-host surfaces may germinate to produce and actively discharge secondary conidia, which may in turn germinate, producing tertiary conidia. This process of iterative germination increases the capacity of the fungus to reach and infect susceptible hosts (Pell *et al.* 2001). Many insects infected with entomophthoralean species demonstrate behavioural changes and exhibit 'summit disease', climbing to an elevated position before death which favours widespread dispersal of conidia by wind currents. (Roy *et al.* 2006). In species that sporulate on living hosts, dispersal is aided by host movement. Entomophthoralean fungi also produce thick-walled, dormant resting

spores allowing survival through periods when hosts are not present (Hajek 1997; Pell *et al.* 2001).

Cordyceps and *Ophiocordyceps* species commonly infect hosts that are hidden in soil, leaf litter or decaying wood (Sung *et al.* 2007a). After host death, the cadaver is colonised by mycelium and hyphal bodies forming a dormant, sclerotium-like resting stage (Evans 1989). This stage allows survival of adverse environmental conditions and may function to synchronise the fungus with the seasonal appearance of a specific host (Hywel-Jones 2004). When conditions are favourable, spores are produced from phototropic stromata which emerge from the substrate to aid dispersal (Evans 1982). Ascospores are forcibly discharged from asynchronously maturing perithecia which steadily release ascospores over an extended period of time, increasing chances of contact with hosts at low population densities. One or more types of anamorph may also be produced on phototrophic synnemata (Evans 1988). Dry conidia are disseminated by air currents and function as long-distance dispersal units, while conidia coated in mucus are adapted to short range dispersal by rain splash and water run-off from leaves (Evans 1989; Hajek 1997). Mucus coatings may also aid in survival of conidia by providing protection from desiccation or ultraviolet radiation (Evans & Samson 1982a).

In most cases, species of *Beauveria* and *Metarhizium* do not produce a teleomorphic stage in their life cycle and host death is followed by rapid and abundant production of conidia on the cadaver surface. Short periods of infection are correlated with seasonal host availability and environmental conditions, with fungal survival in the soil during unfavourable periods (Evans 1988; Meyling & Eilenberg 2007). Conidia are passively dispersed from freely exposed hosts by air currents and rain splash (Inglis *et al.* 2001; Shah & Pell 2003). Meyling *et al.* (2006) showed that conidia of *B. bassiana* could be distributed by the activity of vector insects on plant surfaces. Other arthropods such as collembolans (Dromph & Vestergaard 2002) and mites (Renker *et al.* 2005) have also been shown to act as vectors for dispersal of *Beauveria* and *Metarhizium* conidia in soil. Formation of hyphal strands or synnemata may also facilitate dispersal from infected hosts which are buried in soil or otherwise hidden (Evans 1982; Keller & Zimmermann 1989).

1.5 Biological control

Biological control (or biocontrol) can be defined as the use of living organisms to suppress the population density or impact of specific pests. Microbial control involves the use of pathogenic microorganisms such as fungi, bacteria or viruses as biocontrol agents (Eilenberg *et al.* 2001). The importance of entomopathogenic fungi as natural regulators of arthropod populations and ability to cause widespread epizootics demonstrates their potential for microbial control of

arthropod pests (Carruthers *et al.* 1991). Although this potential was first explored from the late nineteenth century onwards (Steinhaus 1956, 1975), interest in microbial control declined with the introduction of synthetic chemical insecticides in the 1940s and 1950s (Charnley 1991). The broad activity spectra and residual effects of chemical pesticides were initially considered to be desirable properties but are now seen as detrimental due to adverse effects on non-target invertebrates and development of resistance in target pests (Federici 1999; Kaya & Lacey 2007). These issues, together with increasing concerns over human safety, have led to renewed interest in the use of entomopathogenic fungi for microbial control (see Butt 2002; Charnley & Collins 2007; de Faria & Wraight 2007; Inglis *et al.* 2001; Shah & Pell 2003).

Compared with chemical insecticides, microbial control offers a number of advantages. Environmental benefits include increased safety for humans and domestic animals and reduced contamination of food, soil and groundwater (Lacey *et al.* 2001). The narrow activity spectra of microbial control agents results in increased biodiversity and activity of beneficial invertebrates such as predators and parasitoids, pollinators, and earthworms (Goettel *et al.* 1990; Vestergaard *et al.* 2003). Further advantages include limited development of host resistance and compatibility with other biocontrol agents (Lacey *et al.* 2001). Some of these advantages may also be viewed as disadvantages, especially in terms of practical considerations and commercial marketability. High selectivity may result in the need for additional control measures if more than one major pest is present (Kaya & Lacey 2007). Limited persistence can also become an issue and several applications may be required for successful control (Lacey *et al.* 2001; Lacey & Shapiro-Ilan 2003).

Entomopathogenic fungi have certain advantages over other insect pathogens for arthropod control. Unlike bacteria and viruses, which have a requirement for ingestion, fungi cause infections by direct invasion through the cuticle. This mode of action means they are capable of infecting non-feeding stages such as eggs and pupae (Charnley & Collins 2007) and sap-feeding hemipteran species (Carruthers *et al.* 1991; Payne *et al.* 1988). Fungi may also be the best choice for microbial control of coleopteran pests, which have few associated bacterial or viral pathogens (Samson *et al.* 1988).

Entomopathogenic fungi can be employed under four biological control strategies as defined by Eilenberg *et al.* (2001): (1) classical; (2) inoculation; (3) inundation; and (4) conservation. Any one of these strategies may be more suitable for a particular pest problem or habitat. Each approach also has its own requirements for pathogen characteristics such as specificity, virulence and persistence (Fuxa 1987).

Classical biological control involves the introduction and permanent establishment of an exotic species/strain with the aim of providing long term control (Eilenberg *et al.* 2001). Generally this approach is used for control of an invasive (exotic) arthropod that has become established and reached pest status in the absence of its normal natural enemies (Hajek *et al.* 2007a). A suitable pathogen is identified from the areas of pest origin and released into the new area where the pest needs to be controlled (Shah & Pell 2003). Where possible strains are selected from areas with similar climatic conditions to the release sites (*e.g.* Milner *et al.* 1982). Pathogens chosen for introduction should be highly adapted to their target host with little capacity for infection of non-target species (Hajek & Goettel 2007). For successful establishment the pathogen must be capable of long-term survival within the host population or external environment (Hajek *et al.* 2007a; Payne *et al.* 1988). Nineteen fungal species have been released in classical biological programmes for insect pests, with *Metarhizium anisopliae* and entomophthoralean species the most commonly used. Most introductions of *M. anisopliae* were undertaken early in the last century and their impact has not been widely determined (Hajek *et al.* 2007b). Results of programmes using entomophthoralean species have been more reliably documented and establishment and successful control has been demonstrated in several cases (Pell *et al.* 2001; Hajek *et al.* 2007b). Entomophthoralean fungi have several biological characteristics which contribute to their effectiveness as classical biocontrol agents and these include strict host specificity, potential for epizootics, and persistence due to the formation of resting spores (Hajek *et al.* 2007a).

Inoculation and inundation biocontrol have often been included under the category of augmentation. Inoculative releases are expected to reproduce and spread after application but are not required to become permanently established. Pest control is only temporary and additional applications will eventually be required (Fuxa 1987; Eilenberg *et al.* 2001). In contrast, inundation biological control relies directly on the action of the released individuals which are not expected to multiply or persist in the environment (Eilenberg *et al.* 2001), and in this way is similar to the use of a chemical insecticide (Shah & Pell 2003). In practice the distinction between these two strategies is not always clearly defined; pathogens released as innundative agents may have the potential for multiplication resulting in residual (*i.e.* inoculative) effects (Hajek 2004; Chandler *et al.* 2008).

Inundation is the most widely used strategy for microbial control of arthropod pests. Fungal pathogens used in this way are mass produced, and their infectious propagules are formulated as mycoinsecticides (Inglis *et al.* 2001; Butt 2002; Shah & Pell 2003). De Faria & Wraight (2007) list 129 mycoinsecticides and mycoacaricides currently undergoing registration or available

worldwide. At least 12 fungal species (or subspecies) have been employed as mycoinsecticides, with the majority being hypocrealean anamorphs. *Beauveria bassiana*, *Isaria fumosorosea*, *Lecanicillium* spp, and *Metarhizium anisopliae* are the most commonly used taxa (Charnley & Collins 2007; de Faria & Wraight 2007). Products marketed as mycoacaricides have been based solely on the acarine pathogen *Hirsutella thompsonii*, although a number of products intended for insect control also claim to control mites (de Faria & Wraight 2007). Entomophthoralean fungi have shown limited application for mycoinsecticide development, mainly due to difficulties in mass-production and formulation (Milner 1997; Pell *et al.* 2001; Shah & Pell 2003).

Conservation biological control differs from other strategies in that natural enemies are not released into the pest population. Instead, farming practices or environmental manipulations are used to enhance the activity of specific natural enemies (Fuxa 1987) or previously released biocontrol agents (Eilenberg *et al.* 2001; Pell *et al.* 2001). Cultural practices to encourage entomopathogenic fungi can include measures such as increasing moisture *e.g.* by irrigation or increased canopy cover, reducing tillage, and reducing fungicide applications (Fuxa 1987; Hummel *et al.* 2002). Managed field margins or non-crop areas (*e.g.* weed strips) may act as effective reservoirs for fungal pathogens which could then reduce pests in adjacent crops. Research on conservation biological control using fungal entomopathogens has mainly focused on aphid pathogenic Entomophthorales (Baverstock *et al.* 2008; Ekesi *et al.* 2005; Shah & Pell 2003). However, the potential importance of *B. bassiana* and *M. anisopliae* for conservation biological control in agricultural systems has also been recognised (Meyling & Eilenberg 2006, 2007).

Research on the diversity of entomopathogenic fungi in natural habitats may have considerable application in biological control programmes. A direct outcome of such studies is the isolation of novel strains for screening against target pests. Most fungi used for arthropod control have originated from strains isolated from agricultural ecosystems (Hywel-Jones 2002). However, natural forests have a much greater associated fungal diversity and many entomopathogenic species are naturally restricted to these habitats (Evans 1974; Hywel-Jones 2002). Natural habitats may be associated with increased genotypic diversity in ubiquitous species such as *M. anisopliae* and *B. bassiana*. Bidochka *et al.* (2001, 2002) showed that unique genotypes of both species present in Canadian forests were not found in agricultural habitats. Native forests may therefore represent an important and relatively unexplored source of entomopathogenic fungi for biological control.

Concerns have been raised over the potential for adverse ecological impacts from the importation of exotic fungi for biological control (*e.g.* Lockwood 1993a, 1993b). Use of native fungal strains is less likely to have unwanted environmental consequences and involves fewer regulatory constraints for commercial development compared with exotic pathogens (Chandler *et al.* 2008; Prior 1992). While infection of non-target arthropods by introduced pathogens is often seen as the most important ecological issue, another aspect which should be considered is the competitive displacement of native entomopathogenic species (Butt 2002; Cook *et al.* 1996; Hokkanen *et al.* 2003; Lockwood 1993a, 1993b). Background knowledge of indigenous fungal diversity is needed to more fully evaluate the environmental impacts of exotic strains introduced for classical or augmentative biocontrol (Chandler *et al.* 2008; Meyling 2008).

1.6 Thesis scope

Entomopathogenic fungi have been infrequently recorded from native forests in New Zealand. The main objectives of this study were to examine the taxonomic diversity of the genera *Beauveria* and *Isaria* in native forests using morphology and molecular identification tools, and to provide a preliminary assessment of the host range and biocontrol potential of representatives from these genera.

CHAPTER TWO: GENERAL METHODS

2.1 Collection areas

Entomopathogenic fungi were collected from native forest at 25 sites around New Zealand. Collection sites are listed in Table 2.1 with districts according to Crosby *et al.* (1976, 1998). Localities were divided into five main regions: Buller/Westland; North Canterbury; Nelson/Tasman; Tongariro/Rangitikei; and Bay of Plenty. Most sites consisted of mixed podocarp/broadleaf forest, although some collections were also made from mixed podocarp/broadleaf/beech and pure beech forests.

Table 2.1 Collection sites.

Locality	Forest type	Crosby district
Cascade Valley, Haast	Podocarp/broadleaf	Westland
Kahikatea Swamp Forest Walk, Haast	Podocarp/broadleaf	Westland
Hapuka Estuary Walk, Okuru	Podocarp/broadleaf	Westland
Terrace Walk, Franz Josef	Podocarp/broadleaf	Westland
Lake Kaniere Walkway, Hokitika	Podocarp/broadleaf	Westland
Goldsborough (Shamrock) Track, Hokitika	Podocarp/broadleaf	Westland
Mount French Track, Lake Brunner	Podocarp/broadleaf/beech	Buller
Nile River Valley Walk, Charleston	Podocarp/broadleaf	Buller
Pororari River Track, Punakaiki	Podocarp/broadleaf	Buller
Truman Track, Punakaiki	Podocarp/broadleaf	Buller
Charming Creek Walkway, Westport	Podocarp/broadleaf	Nelson
Oparara Arch Walk, Karamea	Podocarp/broadleaf/beech	Nelson
Nikau Loop Walk, Karamea	Podocarp/broadleaf	Nelson
Rolling Creek, Wangapeka Valley	Podocarp/broadleaf/beech	Nelson
Eves Valley Scenic Reserve, Brightwater	Podocarp/broadleaf/beech	Nelson
Snowdens Bush Scenic Reserve, Brightwater	Podocarp/broadleaf/beech	Nelson
Loop Track, Lake Rotoiti	Podocarp/broadleaf/beech	Nelson
Wooded Gully Track, Mount Thomas	Beech	North Canterbury
Devils Punchbowl Track, Arthurs Pass	Beech	North Canterbury
Mangawhero Falls Walk, Mount Ruapehu	Podocarp/broadleaf	Taupo
Mangawhero Forest Walk, Mount Ruapehu	Podocarp/broadleaf	Taupo
Old Blyth Track, Mount Ruapehu	Podocarp/broadleaf	Taupo
Paengaroa Scenic Reserve, Mataroa	Podocarp/broadleaf	Rangitikei
Aongatete Short Loop Track, Katikati	Podocarp/broadleaf	Bay of Plenty
Lindemann Loop Track, Katikati	Podocarp/broadleaf/beech	Bay of Plenty

2.2 Fungal collection and isolation

Infected insect specimens were generally collected in 20 ml plastic containers lined with dry tissue paper that were sterilized by autoclaving before use. Large specimens were collected in paper bags or sterile whirlpak bags. In most cases specimens were stored at 4°C for up to a week before isolation of cultures. Where possible, hosts were identified according to Crowe (2002); Clapperton *et al.* (1989); Larivière (1996); Larivière et al. (2006); and Holloway (1956). Specimens were examined under a dissection microscope to confirm fungal infection. Several specimens could not be reliably identified to any arthropod group. Often these were small larval stages or in an advanced state of decomposition and lacking any readily identifiable features.

For preliminary identification of fungal species, conidiogenous structures were mounted in lactic acid or 0.03% lactofuchsin and examined at 600× magnification. Isolations were made onto standard 90cm plates of dilute Sabouraud dextrose yeast agar (dSDYA: 4 g/L dextrose; 1 g/L peptone; 1 g/L yeast extract; 15g /L agar) supplemented with 250 mg/ml streptomycin sulphate and 50 mg/ml chlortetracycline hydrochloride. For isolations, a flamed inoculating needle was used to cut a small (approximately $1mm^3$) cube of agar which was gently wiped over conidiophores to pick up conidia. Conidia were inoculated at four equidistant points on each of two or three plates and incubated at 20°C. Cultures were examined daily to confirm germination and check for the development of contaminating fungi. If necessary, cultures were transferred to fresh plates of dSDYA amended with antibiotics as above.

Pure cultures were stored as agar plugs in 10% glycerol frozen at -80°C and in sterile distilled water at 4°C. All isolates are stored in the University of Canterbury fungal culture collection. For routine use, stock cultures were prepared in dSDYA slopes and stored at 4°C.

2.3 DNA extraction

For DNA extraction a loopful of conidia from pure cultures was spread with a glass spreader over plates of potato dextrose agar (PDA) overlaid with sterile colourless cellophane. Plates were incubated for three to five days at 25°C or until a thin layer of mycelium covered the entire plate. Approximately 100 mg of mycelium was harvested with a flamed spatula into a sterile 1.5 ml Eppendorf tube and stored frozen at -20°C. To extract DNA, fungal mycelium was ground in liquid nitrogen with a sterile plastic pestle and mixed with 500 µl of extraction buffer (0.15M NaCl, 50mM Tris-HCl, 10mM Na_2EDTA, 3% sodium dodecyl sulphate). Tubes were incubated at 65°C in a heating block for 40-60 minutes with periodic mixing by inversion. The solution was then mixed with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and

centrifuged at 13000 rpm for 10 minutes. The aqueous layer was removed and extracted again with one volume of chloroform-isoamyl alcohol (24:1). Following centrifugation as above, the aqueous layer was removed and DNA was precipitated by addition of an equal volume of ice-cold isopropanol and centrifuging at 10000 rpm for 10 minutes. Pellets were washed twice in 500 µl 70% ethanol and air-dried at 37°C. DNA was resuspended in 50 µl of molecular biology grade water and stored frozen at -20°C.

Extraction of *Isaria* cultures yielded sticky gel-like pellets at the final DNA precipitation stage and these were subjected to a further clean-up stage to remove co-precipitated polysaccharides and provide suitable DNA for subsequent PCR. Seventy-five microlitres of 5M NaCl was added and mixed, and 60 µl of cetyltrimethylammonium bromide (CTAB) solution (10% w/v CTAB in 0.7M NaCl) was added and mixed again. The suspension was incubated at 65°C for 20 minutes and centrifuged for two minutes at 8000 RPM. The supernatant was transferred to a new tube and DNA was precipitated, washed and suspended as above.

2.4 PCR amplification and sequencing

Three nuclear gene regions were amplified by PCR and sequenced in this study. The entire ITS1-5.8S-ITS2 region was amplified using the primer pairs TW81/AB28 (Curran *et al.* 1994) or ITS5/ITS4 (White *et al.* 1990). An approximately 330 base pair fragment of the β-tubulin gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). An approximately 530 base pair fragment from the EF1-α gene was amplified using the primers 1577F and 2218R (Rehner & Buckley 2005). All primer sequences are shown in Table 2.2.

Table 2.2 PCR and sequencing primers used in this study

Region	Primer	Sequence	Source
ITS	TW81	5' GTTTCCGTAGGTGAACCTGC 3'	Curran *et al.*1994
ITS	AB28	5' ATATGCTTAAGTTCAGCGGGT 3'	Curran *et al.* 1994
ITS	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG 3'	White *et al.* 1990
ITS	ITS4	5' TCCTCCGCTTATTGATATGC 3'	White *et al.*1990
β-tubulin	Bt2a	5' GGTAACCAAATCGGTGCTGCTTTC 3'	Glass & Donaldson 1995
β-tubulin	Bt2b	5' ACCCTCAGTGTAGTGACCCTTGGC 3'	Glass & Donaldson 1995
EF1-α	1577F	5' CARGAYGTBTACAAGATYGGTGG 3'	Rehner & Buckley 2005
EF1-α	2218R	5' CCRAACRGCRACRGTYYGTCTCAT 3'	Rehner & Buckley 2005

All PCR amplifications were performed in a total reaction volume of 25 µl including 0.4 mM of each primer, 200 mM dNTPs, 2.5 µl reaction buffer, 2.5 mM $MgCl_2$, 2 µl template DNA and 0.7U Taq Polymerase. Generally a 1/100 dilution of the extracted DNA solution was used as

template DNA for PCR although in some cases a 1/10 dilution or undiluted sample was used. Positive (DNA) and negative (sterile water) controls were included for each reaction.

All PCR amplifications were initiated with a 2 minute denaturation step at 96°C and a final extension step of 10 minutes at 72°C. The following specific temperature profiles were used for each primer pair: ITS5/ITS4) denaturation 1 minute at 96°C, annealing 30 seconds at 56°C, 45 seconds extension at 72°C (30cycles); Bt2a/Bt2b) denaturation 1 minute at 96°C, annealing 30 seconds at 58°C, 45 seconds extension at 72°C (30 cycles); 1577F/2218R) denaturation 1 minute at 96°C, annealing 30 seconds at 55°C, 1 minute extension at 72°C (30 cycles). PCR products were visualised by ethidium bromide staining following electrophoresis of 5 µl of each product in 1% agarose gels. PCR products were cleaned using a commercial cleanup kit and sequenced in both forward and reverse directions (using the reaction primers) at AWCGS Sequencing Facility, Massey University; or Canterbury Sequencing, University of Canterbury. Consensus sequences were assembled from forward and reverse sequences using ChromasPro version 1.34.

2.5 Phylogenetic analyses

For each dataset sequences were aligned with ClustalW in MEGA version 4.0 (Tamura *et al.* 2007) using the default parameters and improved manually if necessary. Phylogenetic analyses were conducted using neighbour-joining (NJ), maximum parsimony (MP) and Bayesian inference methods. NJ and MP analyses were performed with MEGA version 4.0 (Tamura *et al.* 2007). MP analyses were conducted using the close-neighbour-interchange algorithm (Nei & Kumar 2000) with a search level of three, in which the initial trees were obtained from the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option) in NJ and MP analyses. Support for each branch was obtained from bootstrap analysis (Felsenstein 1985) using 1000 replicates. Phylogenetic analysis using Bayesian inference was conducted using MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Models of nucleotide substitution that best fitted each dataset were selected using the Akaike Information Criterion in MrModelTest version 2.0 (Nylander 2004) implemented in PAUP_4.0b10 (Swofford 2002). For each dataset MrBayes was run in two simultaneous, independent analyses. Each analysis was run with four chains (three cold, one heated) for 2 000 000 generations, saving trees every 100 generations (including the first generation) to give a total of 20 001 trees. The first 25% of the trees were discarded as "burn-in" to allow the log-likelihood scores to become stable. A 50% consensus was generated from the remaining trees with support values representing the posterior probabilities.

CHAPTER THREE: THE GENUS *BEAUVERIA* IN NATIVE FORESTS

3.1 Introduction

Beauveria is one of the most commonly encountered genera of entomopathogenic fungi, due to its global distribution, broad host range, and frequent occurrence in a range of habitats (Rehner 2005). Since the early discovery of their ability to cause disease in insects (see Major 1944; Steinhaus 1956, 1975), *Beauveria* species have been widely investigated as biocontrol agents and currently form the basis of several commercially available mycoinsecticides (de Faria & Wraight 2007). However, despite a long history of research, taxonomy in the genus has often been problematic, due to a lack of stable and informative morphological characters that can be used to delineate species.

Following the discovery by Bassi of the fungal nature of the 'mark' or 'muscardine' disease of silkworms, in 1835 Balsamo-Crivelli formally named the causative pathogen as *Botrytis paradoxa*, later changing this to *Botrytis bassiana* in honour of Bassi (Steinhaus 1956, 1975). Several species with comparable morphology were subsequently described from infected insects, with a tendency for European mycologists to refer their species to *Botrytis* while American workers included similar species in *Sporotrichum*. It was later recognized that *Botrytis bassiana* did not fit well into either of these genera based on the mode of spore development and in 1912 Vuillemin transferred the species to the new genus *Beauveria*, characterised by production of conidia on geniculate, sympodially proliferating conidiogenous cells (de Hoog 1972; Petch 1926).

Further species were described in *Beauveria* based mainly on minor differences in cultural characteristics such as growth rate, amount of sporulation, colony appearance and medium colouration. Petch (1926) examined several representative isolates and recorded spore dimensions and colony characteristics on a range of media. Petch found that cultural characters were of little taxonomic value as they were highly variable and could be affected by repeated subculturing and media composition. He concluded that the only major difference between the eight species recorded at that time was the shape of the conidia and recognised only two species: *B. bassiana* with globose spores and *B. densa* with oval spores. By the time MacLeod (1954) published his monograph of *Beauveria* a total of 16 species had been described in the genus. MacLeod followed Petch (1926) in recognising only two species, but used the name *B. tenella* instead of *B. densa*. De Hoog (1972) examined the type specimens of *B. tenella* and *B. densa* and regarded these as *B. bassiana*, concluding that *B. brongniartii* was the correct name for the oval-

spored species. He also accepted a third species, *B. alba*, although this was later transferred to *Engyodontium* (de Hoog 1978).

Additional species of *Beauveria* were recognised on the basis of their distinctive conidial morphology and included several species from South America. De Hoog & Rao (1975) described *B. vermiconia* with comma-shaped conidia from volcanic ash in Chile. *Beauveria velata*, characterised by ellipsoidal, verrucose conidia, was described from infected lepidopteran larvae in Ecuador by Samson & Evans (1982). A species with curved, cylindrical conidia from infected Coleoptera collected in Brazil, although otherwise identical to the previously described *Isaria amorpha,* was shown to produce conidiogenous cells typical of *Beauveria.* Following examination of the type material of *I. amorpha* and a similar species named by Petch (1933) as *Isaria orthopterorum*, both species were recombined as *B. amorpha* (Samson & Evans 1982). An isolate from Scottish moorland soil with similar, smaller conidia was described as the new species *B. caledonica* (Bisset & Widden 1988). The most recently characterised species, *B. malawiensis,* was isolated from a coleopteran larva in Africa and was distinguished by straight cylindrical conidia, globose conidiophores, and pink colouration of hyphae and conidia (Rehner *et al.* 2006a).

Von Arx (1986) claimed to have observed sympodial proliferation of conidiogenous cells in *Tolypocladium* and transferred several members of the genus to *Beauveria*, although this decision was not generally accepted (*e.g.* Gams *et al.* 1998; Samson *et al.* 1988; Sigler *et al.* 1987). Scanning electron microscope examination confirmed that the mode of conidiogenesis in *Tolypocladium* was phialidic and that the genus should be maintained as separate from *Beauveria* (Sigler *et al.* 1987). The distinction between the two genera has also been supported by biochemical (Kadlec *et al.* 1994; Mugnai *et al.* 1989; Todorova *et al.* 1998) and molecular data (Hegedus & Khachatourians 1996; Hodge 1998; Rakotonirainy *et al.* 1991; Stensrud *et al.* 2005; Sung *et al.* 2007a).

The first suggestion of a teleomorphic state for *Beauveria* was made in the mid-nineteenth century by Tulasne who speculated that *B. bassiana* may have an associated *Sphaeria* (*Cordyceps*) teleomorph (Gray 1858). An unnamed teleomorph of *B. bassiana* was reported by Schaerffenberg in 1955, although few details were given and this report has been largely ignored (de Hoog 1972; Huang *et al.* 2002; Li *et al.* 2001). Booth (1961) described a *Beauveria* anamorph for *Pseudeurotium bakeri* (Pseudeurotiaceae). Although superficially similar to *Beauveria*, *Pseudeurotium* anamorphs differ in several characteristics and have since been classified in the genus *Teberdinia* (Sogonov *et al.* 2005). Later authors (von Arx 1986; Samson

et al. 1988) again suggested that the teleomorphs of *Beauveria* were probably *Cordyceps* species. Experimental proof of this connection was first provided by Shimazu *et al.* (1988) who described *Cordyceps brongniartii* which developed on lepidopteran larvae that had been infected with *Beauveria brongniartii.* Single ascospore isolations from the *Cordyceps* stromata were shown to produce the *B. brongniartii* anamorph. Similarly, Li *et al.* (2001) linked *Beauveria bassiana* with the new species *Cordyceps bassiana.* Both associations were later supported by molecular evidence (Huang *et al.* 2002; Liu *et al.* 2002; Rehner & Buckley 2005; Sasaki *et al.* 2007). Molecular analyses have also identified *Cordyceps scarabaeicola* and *Cordyceps staphylinidicola* as teleomorphs of *Beauveria* (Rehner & Buckley 2005; Sung *et al.* 2001; Sung *et al.* 2007a).

Host range in *Beauveria* varies according to species. *Beauveria. bassiana* displays an extremely wide host range and has been recorded from over 700 species in 15 insect orders (Li 1988). The species is also known to commonly infect mites (Chandler *et al.* 2000; Li 1988) and (more rarely) spiders (Petch 1931). While individual isolates often vary in virulence towards different insects (Fargues & Remaudière 1977; Goettel *et al.* 1990; Prior 1992), it remains unclear whether these differences can be interpreted as host-specificity. Recent studies have suggested that strains of *B. bassiana* show little adaptation towards particular hosts and that the species should be regarded as a generalist entomopathogen (Bidochka *et al.* 2002; Kouvelis *et al.* 2008; Rehner & Buckley 2005; Rehner *et al.* 2006b; Uma Devi *et al.* 2008).

Beauveria amorpha also has a relatively wide host range and although more commonly associated with coleopteran hosts has also been recorded from Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera (Hywel-Jones 2004; Petch 1933; Samson & Evans 1982; Rehner & Buckley 2005). Other *Beauveria* species appear to have stricter host preferences. *B. brongniartii* mainly infects Coleoptera, especially members of Scarabaeidae (Fargues & Remaudière 1977; Neuveglise 1994; Rehner & Buckley 2005; Zimmermann 2007a), and it has been suggested that records of this species from other insects may be misidentified (Vestergaard *et al.* 2003). *B. velata* and *B. malawiensis* have only been recorded from lepidopteran and coleopteran hosts, respectively (Rehner *et al.* 2006a; Samson & Evans 1982), although both species have not been collected extensively enough to allow any reliable conclusions on the extent of their host range. *B. vermiconia* was originally isolated from volcanic ash (de Hoog & Rao 1975) and Mugnai *et al.* (1989) speculated that the species may represent a primitive member of the genus that has not yet evolved an entomopathogenic life cycle. However, *B. vermiconia* was later found to naturally infect a coleopteran species in Chile, and pathogenicity towards a related species was also demonstrated in laboratory bioassays (Glare *et al.* 1993a). Similarly, *B. caledonica*, first

described from a soil isolate (Bisset & Widden 1988), was subsequently found as a pathogen of several coleopteran species (Glare *et al.* 2008; Kirchsner 2001; Reay *et al.* 2008; Rehner & Buckley 2005).

Although the distinctive conidiogenous cells of *Beauveria* are easily recognised, conidial form is the only morphological feature useful for species-level identification. This has often led to difficulties in routine identification of *Beauveria* isolates, especially with the two most common species, *B. bassiana* and *B. brongniartii*. While the two species have been traditionally separated by conidial size and shape, these characters are highly variable and have been shown to be particularly influenced by cultural conditions (Mugnai *et al.* 1989; Townsend *et al.* 1995). The importance of *Beauveria* species in insect biocontrol programmes and the limitations of using morphological criteria for identification have led to increasing efforts to find alternative methods of characterization in the genus (Glare 2004).

Initial attempts to find additional characters for species recognition in *Beauveria* used biochemical or chemotaxonomic approaches. Mugnai *et al.* (1989) examined carbohydrate utilisation and enzyme production patterns in several *Beauveria* species and generally found that species could be separated based on their biochemical profiles. A similar approach using commercial carbohydrate utilization (API) strips was able to separate *Beauveria* from other entomopathogenic fungi but could not reliably discriminate between species in the genus (Rath *et al.* 1995; Todorova *et al.* 1998). *Beauveria* species are known to produce diverse secondary metabolites (Isaka *et al.* 2005), although their utility as taxonomic markers has not been widely evaluated. Based on beauveriolide production, Kadlec *et al.* (1994) were able to distinguish *Beauveria* from *Tolypocladium* but could not differentiate species within *Beauveria*.

A large number of studies have examined genetic variation in *Beauveria*. Most investigations have been focused towards biocontrol and have mainly included isolates of *B. bassiana* or *B. brongniartii*, often from a single host or geographic origin. The first attempts at genetic characterisation of *Beauveria* were based on isozyme analysis (*e.g.* Poprawski *et al.* 1988; McCoy & Boucias 1989; Bridge *et al.* 1990; St Leger *et al.* 1992a) which uses the electrophoretic separation of enzyme polymorphisms to detect genetic variation (Micales *et al.* 1986). This technique was later superseded by a range of PCR-based methods that instead examine DNA directly. Random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) analysis have been widely used to differentiate *Beauveria* isolates (*e.g.* Bidochka *et al.* 1994; Castrillo *et al.* 1999, 2003; Glare & Inwood 1998; Luz *et al.* 1998; Maurer *et al.* 1997; Neuveglise *et al.* 1994; Piatti *et al.* 1998; Urtz & Rice 1997). However, both

methods have several associated disadvantages (Glare 2004; McDonald 1997; Meyling 2008) and their use has been largely replaced by newer techniques including DNA sequencing (*e.g.* Glare *et al.* 2008; Kouvelis *et al.* 2008; Reay *et al.* 2007, 2008; Rehner *et al.* 2006a, 2006b; Rehner & Buckley 2005), amplified restriction length polymorphism (AFLP) (Cruz *et al.* 2006; Aquino de Muro *et al.* 2003, 2005; Hadapad *et al.* 2006; Uma Devi *et al.* 2006) and microsatellite-based markers (Enkerli *et al.* 2001; Estrada *et al.* 2007; Rehner & Buckley 2003; Takatsuka 2007; Wang *et al.* 2005).

Few studies have used molecular techniques to specifically resolve taxonomic or phylogenetic questions within *Beauveria*. The most significant recent contribution to the understanding of species concepts in the genus has been a phylogenetic analysis based on ITS and EF1-α sequences (Rehner & Buckley 2005). The authors examined a set of exemplar isolates largely representing worldwide diversity of *Beauveria* species (although New Zealand isolates were excluded). While sequence data generally confirmed the existing morphologically based classifications in the genus, a major finding of the study was that *B. bassiana* isolates could be separated into two genetically distinct, but morphologically indistinguishable lineages. One lineage (designated as 'Clade A' or '*B. bassiana s.l.*') was found to have a global distribution, while the other group ('Clade C' or 'pseudobassiana') was less common and restricted to Europe and North America. Further phylogenetic diversity within *B. bassiana s.l.* indicated that this may also be a species complex with several discrete lineages, corresponding in some cases with geographic origin. Additional evidence for phylogeographic structuring of *B. bassiana s.l.* populations was provided from analysis of two highly variable nuclear intergenic regions EFutr and Bloc (Rehner *et al.* 2006b).

Beauveria was first reported from New Zealand in the late nineteenth century when a fungus infecting lepidopteran larvae was identified as *Sporotrichum globuliferum* (Anon 1893), an earlier synonym of *B. bassiana* (de Hoog 1972). Subsequent New Zealand records have listed *Beauveria* species from over 40 host species in several insect orders (see Table 3.1). However, few reports have included morphological descriptions and taxonomic research has been limited to isolates from agricultural and forestry pests, mainly in Coleoptera (*e.g.* Glare & Inwood 1998; Glare *et al.* 2008; Reay *et al.* 2007, 2008; Townsend *et al.* 1995; Willoughby *et al.* 1998).

Glare & Inwood (1998) and Townsend *et al.* (1995) examined the morphology of *Beauveria* strains isolated from several coleopteran species in New Zealand. Isolates were identified as *B. bassiana* or *B. brongniartii* based on spore dimensions. However, it was noted that some strains producing ellipsoidal conidia on the host and initially identified as *B. brongniartii* only produced

Table 3.1 Records of *Beauveria* species infecting insects in New Zealand.

Species	Host order	Host family	Reference
B. bassiana	Coleoptera	Cerambycidae	Edwards 1965
		Curculionidae	Barker & Addison 1989; Barker *et al.* 1991; Glare & Inwood 1998; Willoughby *et al.* 1998; Reay *et al.* 2007, 2008
		Coccinellidae	Cameron & Walker 1989
		Chyrysomelidae	Glare *et al.* 1993b
		Scarabaeidae	Barker *et al.* 1991; Glare *et al.* 1993b; Townsend *et al.*1995; Glare & Inwood 1998
	Diptera	Syrphidae	Barker *et al.*1991
	Hemiptera	Cicadidae	Anon 2001-2009 [PDD]
		Diaspididae	Ferguson & Fletcher 1991
	Hymenoptera	Ichneumonidae	Anon 2001-2009 [PDD]
		Vespidae	Wigley & Dhana 1988 [a]; Barker *et al.* 1991; Harris *et al.* 2000; Anon 2001-2009 [PDD]
	Lepidoptera	Coleophoridae	Pearson 1989
		Crambidae	Anon 2001-2009 [b]; Glare *et al.* 1993b
		Geometridae	Anon 2001-2009 [ICMP]
		Hepialidae	Helson 1962 [a]; Grehan 1982; Grehan & Wigley 1984
		Noctuidae	Anon 2001-2009 [b]; Helson 1965
		Pyralidae	Mercer 1981 [a]; Anon 2001-2009 [PDD]
		Sesiidae	Scott 1975; Baker 1981
		Saturniidae	Close 1956; Anon 2001-2009 [PDD]
		Tineidae	Anon 2001-2009 [PDD]
		Tortricidae	Hill *et al.* 1985 [b]
B. brongniartii	Coleoptera	Brentidae	Anon 2001-2009 [PDD]
		Carabidae	Anon 2001-2009 [PDD]
		Cerambycidae	Anon 2001-2009 [PDD]
		Scarabaeidae	de Hoog 1972; Townsend *et al.* 1995; Glare & Inwood 1998
	Hemiptera	Cicadidae	Laird 1991[a]
	Hymenoptera	Vespidae	Laird 1991 [a]
	Lepidoptera	Plutellidae	Anon 2001-2009 [PDD]
		Tortricidae	Anon 2001-2009 [PDD] [c];
	Orthoptera	Tettiigoniidae	Anon 2001-2009 [PDD]
	Phasmatodea	Phasmatidae	Laird 1991[a]
B. caledonica	Coleoptera	Curculionidae	Glare *et al.*2008; Reay *et al.* 2007, 2008

[a]As cited in Glare *et al.*1993b (original reference unavailable); [b] Recorded as '*B. densa*'; [c] Recorded as '*B. tenella*'; PDD = New Zealand Fungal Herbarium; ICMP = International Collection of Micro-organisms from Plants.

spherical conidia in culture. RAPD and ITS-RFLP analyses placed these in a group of New Zealand isolates of *B. bassiana* which were genetically distinct from the overseas material studied. Other *B. bassiana* isolates from New Zealand clustered in a heterogeneous group that included overseas isolates of *B. bassiana* and *B. brongniartii* (Glare *et al.* 1996a; Glare & Inwood 1998). These groupings were also largely supported by analysis of ITS sequences (Glare 2004). It was suggested that the two genetic groups of *B. bassiana* in New Zealand may represent a group of ancient, endemic strains and a group of exotic strains imported after European colonization (Glare *et al.* 1996a, Glare & Inwood 1998). Clearly, the phylogenetic relationships of New Zealand *B. bassiana* isolates need to be evaluated with respect towards current understanding of cryptic diversity in the taxon.

Beauveria brongniartii has been recorded from several different orders of insects in New Zealand (Table 3.1). As *B. brongniartii* is generally considered to be restricted to Coleoptera (Rehner & Buckley 2005; Vestergaard *et al.* 2003), the species may have adapted towards a wider range of hosts in New Zealand. Alternatively, records of *B. brongniartii* in this country from non-coleopteran hosts may be misidentified and could represent one or more morphologically similar species. Dingley (in Anon 2001-2009; Hill *et al.* 1985) also identified *B. densa* from several lepidopteran species. Glare *et al.* (1993b) suggested that records of this species in New Zealand probably referred to *B. bassiana.* Two additional *Beauveria* species have also been recently recorded in New Zealand: *B. caledonica* from Coleoptera and *B. malawiensis* from pine forest soil (Glare *et al.* 2008; Reay *et al.* 2008). Currently there is little knowledge of the host range and distribution of these species in this country.

A number of studies have examined the potential of *Beauveria* species for biocontrol of insect pests in New Zealand. The first investigations were carried out as early as the eighteen-nineties when a commercial preparation of '*Botrytis tenella*' (probably *Beauveria brongniartii*) was imported from France for the control of codling moth (Anon 1892). More recent studies have focused on pests in habitats where the use of chemical insecticides is not sustainable for economic and/or environmental reasons. Biological control is considered an particularly attractive option in pasture where large-scale use of pesticides is not practical (Willoughby *et al.* 1998). Coleopteran species are among the most serious pasture pests in New Zealand and are known to cause major economic losses (Barlow & Goldson 2002; Goldson *et al.* 2005). *Beauveria bassiana* isolates have shown promise for control of several coleopteran pasture pests including the native *Costelytra zealandica* (Goh *et al.* 1991b; Townsend *et al.* 1995) and invasive species *Listronotus bonariensis* (Barker *et al.* 1991; Goh *et al.* 1991a) and *Sitona lepidus* (Brownbridge *et al.* 2006; Nelson *et al.* 2004; Willoughby *et al.* 1998). *Beauveria*

species have also been evaluated for biological control of coleopteran pests in New Zealand production forests. *Beauveria caledonica* was recently discovered infecting two introduced bark beetle species, *Hylastes ater* and *Hylurgus ligniperdus*, which are pests of *Pinus radiata* forests (Glare *et al.* 2008). Current research is assessing the role of *B. caledonica* and other *Beauveria* species in the natural regulation of bark beetle populations in these habitats (M. Brownbridge pers. comm. 2008; Reay *et al.* 2008).

As conservation areas, native forests are another habitat where biological control may provide the most sustainable approach for managing invasive insects. Of several introduced social wasp species that have become established in New Zealand, *Vespula vulgaris* and *V. germanica* have had the most severe ecological impact on native fauna, through both competition and direct predation (*e.g.* Beggs 2001; Beggs & Wilson 1991; Thomas *et al.* 1990; Toft & Rees 1998). Although two wasp parasitoids were introduced for classical biological control, one failed to establish (Beggs *et al.* 2002) while the other has had no significant effect on *Vespula* populations (Beggs *et al.* 2008). Currently wasp control programmes are reliant on insecticide baits which may have undesirable non-target effects and only have limited application considering the large areas where control needs to be achieved (Beggs *et al.* 1998; Harris & Etheridge 2001; Harris & Rees 2000). Harris *et al.* (2000) demonstrated that adults and larvae of *V. vulgaris* were highly susceptible to infection by *B. bassiana* in laboratory bioassays. However, only a limited number of isolates were tested in this study, and the authors suggested additional screening to identify virulent strains for further evaluation as biocontrol agents.

In this chapter, morphological and molecular data was used to investigate the taxonomic diversity of *Beauveria* isolates from invertebrate hosts in native forests. In addition, insect bioassays of two *Beauveria* species were conducted to examine host specificity and to identify isolates which may have potential for biocontrol of *Vespula* wasps.

3.2 Methods

3.2.1 Morphological characterisation

Beauveria species were collected and isolated from native forest as described in chapter two. Fungi from infected arthropods and corresponding cultures were identified as *Beauveria* based on their conidiogenous structures *i.e.* conidiogenous cells with a swollen base and a slender geniculate, denticulate rachis, typically arranged in dense clusters to form characteristic 'spore balls' (de Hoog 1972). An additional culture (T875, provided by N. Waipara) was also examined. For species determination, measurements were made from conidia taken directly from infected hosts and from cultures grown at 25°C for 14-21 days in darkness on ¼ strength Sabouraud dextrose yeast agar (¼SDYA: Goettel & Inglis 1997). For each specimen and culture, length and width of 25 conidia were measured. Conidia were mounted in lactic acid and examined at 600× magnification. Photomicrographs were taken using a digital camera. For each specimen, 25 measurements of phialide and conidial dimensions were made using ImageJ v.1.40 image analysis software (NIH), calibrated with a photomicrograph taken from a slide micrometer. Species were determined by comparing conidial morphology with the descriptions given by de Hoog (1972), Bissett & Widden (1988), and Rehner *et al.* (2006a). To determine any significant relationship between host affiliation and conidial size within each morphological species, mean conidial sizes were square-root transformed and analysed by ANOVA. Means were separated by Tukeys test. Statistical analyses were performed using SPSS version 11.00.

To determine whether previous identifications of *Beauveria brongniartii* in New Zealand were correct, all specimens of *B. brongniartii* in the PDD herbarium were examined microscopically as above. All specimens of *Beauveria densa* and *Beauveria tenella* in PDD were also examined (Table 3.2).

Table 3.2 *Beauveria* specimens from the PDD herbarium examined in this study.

PDD#	Species	Host	District
26212	*B. brongniartii*	*Cicindela tuberculata* [a] (Coleoptera: Carabidae)	Auckland
32590	*B. brongniartii*	*Cicindela* sp. [a]	Auckland
33075	*B. brongniartii*	*Cicindela* sp. [a]	n.a.
33092	*B. brongniartii*	*Cicindela* sp. [a]	Auckland
34706	*B. brongniartii*	Phasmatodea	Auckland
34707	*B. brongniartii*	Phasmatodea	Auckland
34800	*B. brongniartii*	n.a.	Northland
53437	*B. brongniartii*	*Xuthodes batesi* (Coleoptera: Cerambycidae)	Auckland
70281	*B. brongniartii*	*Caedicia simplex* (Orthoptera: Tettiigoniidae)	Auckland
73898	*B. brongniartii*	n.a.	Auckland
73899	*B. brongniartii*	*Vespula germanica* (Hymenoptera: Vespidae)	Auckland
78373	*B. brongniartii*	*Lasiorhynchus barbicornis* (Coleoptera: Brentidae)	Northland
78377	*B. brongniartii*	*Ericodesma argentosa* (Lepidoptera: Plutellidae	Northland
13960	*B. densa*	Coleoptera	Buller
26260	*B. densa*	*Opogona omoscopa* (Lepidoptera: Tineidae)	Auckland
35494	*B. densa*	*Uresephita maoriales* (Lepidoptera: Crambidae)	Auckland
35523	*B. densa*	*Pseudaletia seperata* [b] (Lepidoptera: Noctuidae)	Northland
25211	*B. tenella*	*Eipiphyas postvittana* [c] (Lepidoptera: Tortricidae)	Wanganui

[a] as "*Neocicindela*"; [b] as "*Pseudoletia*"; [c] as "*Eipiphyas postritana*"; n.a. not available.

3.2.2 Molecular characterisation

To confirm species identifications and examine genetic diversity in New Zealand *Beauveria,* isolates were selected to represent the range of morphological diversity, host affiliations and collection areas. DNA extraction, PCR amplification, sequencing, sequence assembly and alignment methods are described in chapter two. For initial analysis the ITS region was amplified and sequenced using the primers TW81 and AB28. Sequences were aligned with a dataset of *Beauveria s*equences obtained from Genbank (Table 3.3) and chosen to cover a wide range of geographic and phylogenetic diversity within the genus. Based on the phylogeny of Sung *et al.* 2007a, *Cordyceps c.f. takaomontana* (BCC28612) and *Isaria cicadae* (NC25, this study) were chosen as appropriate outgroup taxa. An initial phylogenetic analysis of the whole ITS dataset was conducted using the neighbour-joining method. Based on the results from the NJ analysis, a subset of sequences was selected with representatives from each host group for each significant clade. This dataset was futher analysed using maximum parsimony and Bayesian likelihood inference. To provide further support for the ITS phylogeny, representative isolates were selected for sequencing of part of the EF1-α gene using the primers 2218R and 1577F and analysed as above. All methods used for phylogenetic analysis are detailed in Chapter Two.

To determine if data from both genes could be combined in a single analysis, topological incongruence was examined by comparing bootstrap (BS) and posterior probability (PP) values. Clades that were supported by =>95 % PP or >70 BS were considered to be significantly supported by the data (Reeb *et al.* 2003).

Table 3.3 Genbank sequences included in phylogenetic analyses of *Beauveria* isolates.

Species	Strain	Country	Host	ITS Genbank #	EF1-α Genbank #	Reference
B. bassiana	ARSEF 843	Costa Rica	Lepidoptera: Saturniidae	AY532055	AY531964	Rehner & Buckley 2005
Beauveria amorpha	ARSEF 2641	Brazil	Hymenoptera: Formicidae	AY532008	AY531917	Rehner & Buckley 2005
Beauveria amorpha	ARSEF 1969	Peru	Coleoptera: Curculionidae	AY531998	AY531907	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 300	Australia	Hemiptera: Lygaeidae	AY532015	AY531924	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 326	Australia	Lepidoptera: Pyralidae	AY532021	AY531929	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 1848	Belgium	Coleoptera: Rhizophagidae	AY531995	AY531904	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 652	China	Lepidoptera: Pyralidae	AY532032	AY531941	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 1802	Greece	Hemiptera: Miridae	AY531991	AY531900	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 1153	Morocco	Coleoptera: Curculionidae	AY531975	AY531884	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 1811	Morocco	Coleoptera: Curculionidae	AY531992	AY531901	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 296	USA	-	AY532013	AY531922	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 344	USA	Coleoptera: Chrysomelidae	AY532023	AY531932	Rehner & Buckley 2005
Beauveria bassiana	ARSEF 751	Vietnam	Coleoptera: Chrysomelidae	AY532045	AY531954	Rehner & Buckley 2005
Beauveria cf. bassiana	ARSEF 4933	France	Coleoptera: Scolytidae	AY532029	AY531938	Rehner & Buckley 2005
Beauveria cf. bassiana	ARSEF 3220	Portugal	Lepidoptera: Tortricidae	AY532020	AY531928	Rehner & Buckley 2005
Beauveria cf. bassiana	ARSEF 2054	USA	Lepidoptera: Lymantriidae	AY532002	AY531911	Rehner & Buckley 2005
Beauveria cf. bassiana	ARSEF 3405	USA	Lepidoptera: Lymantriidae	AY532022	AY531931	Rehner & Buckley 2005
Beauveria brongniartii	ARSEF 4362	Japan	Soil	AY532025	AY531934	Rehner & Buckley 2005
Beauveria brongniartii	JE 276	Switzerland	Coleoptera: Scarabaeidae	DQ376245	DQ376244	Rehner *et al.* 2006a
Beauveria caledonica	ARSEF 2567	Scotland	Soil	AY532006	AY531915	Rehner & Buckley 2005
Beauveria caledonica	ARSEF 1567	Switzerland	Coleoptera: Scolytidae	AY531986	AY531894	Rehner & Buckley 2005
Beauveria cf. caledonica	ARSEF 2251	Brazil	Coleoptera	AY532003	AY531912	Rehner & Buckley 2005
Beauveria malawiensis	IMI 228343	Malawi	Coleoptera: Cerambycidae	DQ376247	DQ376246	Rehner *et al.* 2006a
Beauveria vermiconia	ARSEF 2922	Chile	Soil	AY532012	AY531920	Rehner & Buckley 2005
Cordyceps cf. scarabaeicola	EFCC 252	South Korea	-	AY532057	AY531966	Rehner & Buckley 2005
Cordyceps cf. staphylinidicola	ARSEF 7044	Korea	-	AY532040	AY531949	Rehner & Buckley 2005
Cordyceps scarabaeicola	ARSEF 5689	China	Coleoptera: Scarabaeidae	AY532030	AY531939	Rehner & Buckley 2005
Cordyceps cf. takaomontana	BCC28612	Thailand	Lepidoptera	FJ765285	FJ765268	Ridkaew & Luangsa-ard unpublished

3.2.3 Insect bioassays

Tenebrio molitor

To examine host specificity in *B. bassiana* and *B. malawiensis*, strains isolated from various hosts were tested in laboratory bioassays to determine their relative virulence towards a coleopteran species. Strains isolated from Coleoptera, Hemiptera, and Hymenoptera were tested against larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae). This species was used as a model organism because large numbers of larvae were available from a commercial supplier (Biosuppliers Live Insects, Auckland). Eight isolates from each of the host orders above were tested against *T. molitor* in three separate sets of bioassays, with each experiment repeated two times.

All isolates were cultured on PDA for 2-3 weeks at 25°C. Conidia were harvested from plates using a sterile spatula and suspended in 5 ml of sterile aqueous 0.01% Triton X-100. Conidial suspensions were adjusted to 10^6 conidia/ml using a haemocytometer. Conidial viability of all isolates was determined by spreading 100 ul of conidial suspension onto plates of PDA and incubating at 25°C for 20 hours. The germination rate was assessed at 20 hours from counts of 300 conidia (Goettel & Inglis 1997) observed from inverted plates at 100× magnification.

Each bioassay was conducted as a completely randomised design. Batches of six *T. molitor* larvae were first sorted into separate 10 ml plastic containers. For each treatment, five replicate 90 mm petri dishes were lined with filter paper and each inoculated with 1 ml of conidial suspension. One ml of 0.01% sterile Triton X-100 was added to the filter paper in each of two sets of five replicate control plates. Each batch of six larvae was then randomly assigned to a treated petri dish. Plates were placed in sealed polythene bags and incubated at room temperature (21°C) for 12 days. Larval mortality was assessed and recorded every two days. To confirm infection, any dead larvae were transferred to separate petri dishes lined with moist filter paper and incubated at room temperature until sporulation was observed. Each experiment was repeated twice with freshly prepared conidial suspensions and a new supply of *T. molitor* larvae. For each replicate, LT_{50} values (time required to kill half of the larvae) in days were recorded. If necessary, LT_{50} times were estimated from linear interpolation between the two assessment periods that straddled the value of '3 dead' (Glare *et al.* 2008). Cumulative mortality (arcsine-transformed) and LT_{50} (log-transformed) data was analysed by ANOVA. Means of LT_{50} values were separated by Tukeys test to determine significant differences between treatments. All statistical analyses were performed using SPSS version 11.00 (SPSS, Inc.).

Vespula vulgaris

To evaluate the potential of *B. bassiana* and *B. malawiensis* for wasp biocontrol, strains were tested in laboratory bioassays to determine pathogenicity towards *V. vulgaris* larvae. Six strains originally isolated from adult *Vespula* species were selected for testing. Conidial suspensions were prepared as described above but were first diluted to a concentration of 10^8 conidia/ml. One millilitre of conidial suspension was added to an equal volume of 0.1% yeast extract to give a final concentration of 5×10^7 conidia conidia/ml. Conidial viability was determined as above.

Comb material was obtained from *V. vulgaris* nests collected from beech forests in the Nelson area. Adults and pupae were removed and combs separated into sections (replicates) containing 20-25 final instar larvae and placed into separate plastic containers with lids. For each treatment three replicates of 20-25 larvae were prepared. Each larva was inoculated on the head capsule with 5 µl of conidial suspension. Larvae were incubated at 25°C and mortality was assessed every 2-3 days for 10 days. Estimation of LT_{50} times and data analysis was performed as described for *Tenebrio molitor*.

3. 3 Results

3.3.1 *Beauveria* collections

Table 3.4 Total numbers of *Beauveria* specimens collected from different host orders and regions.

	Brunner/ Westland	Nelson/ Tasman	North Canterbury	Bay of Plenty	Tongariro/ Rangitikei	Total
Aranaea			1			1
Blattodea	1		1			2
Coleoptera	8	4	1	6	4	23
Dermaptera		1				1
Diplopoda		1				1
Hemiptera	5	4		3	3	15
Hymenoptera	3	16		13		32
Orthoptera	3	1			1	5
Phasmatodea	1				1	2
Unidentified		3	8		4	15
Total	21	30	11	22	13	97

Table 3.5 Host associations of *Beauveria* species.

	B. bassiana	*B. brongniartii*	*B. caledonica*	*B. malawiensis*
Aranaea	1			
Blattodea	2			
Coleoptera	10		5	8
Dermaptera			1	
Diplopoda		1		
Hemiptera	6		1	8
Hymenoptera	9			23
Orthoptera	3			2
Phasmatodea	1			1
Unidentified	13		1	1
Total	45	1	8	43

Table 3.6 Regional collections of *Beauveria* species.

	B. bassiana	*B. brongniartii*	*B. caledonica*	*B. malawiensis*
Brunner/Westland	7		2	12
Nelson/Tasman	18	1	1	10
North Canterbury	10		1	
Bay of Plenty			1	21
Tongariro/Rangitikei	10		3	0

Table 3.7 *Beauveria* specimens collected and examined in this study. Specimen numbers include collection date as *yy/mm/dd.x*.

Isolate #	Specimen #	Species	Host	Host stage	Locality	Region
NC87	050320.11	*B. bassiana*	Araneae	adult	Wooded Gully Track, Mount Thomas	North Canterbury
NC79	050215.2	*B. bassiana*	*Celatoblatta* sp. (Blattodea: Blattidae)	adult	Wooded Gully Track, Mount Thomas	North Canterbury
NC84	050412.2	*B. bassiana*	*Celatoblatta* sp.	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
NC85	050412.3	*B. bassiana*	Coleoptera	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
NC86	050412.4	*B. bassiana*	Coleoptera	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
NC97	050301.1	*B. bassiana*	Coleoptera	adult	Truman Track, Punakaiki	Brunner/Westland
NC53	050408.6	*B. bassiana*	Coleoptera	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC62	050406.31	*B. bassiana*	Coleoptera	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
E1082	040510.17	*B. bassiana*	Coleoptera: Cerambycidae	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman
NC106	050406.34	*B. bassiana*	Coleoptera: Curculionidae	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
TE833	020506.1	*B. bassiana*	Coleoptera: Elateridae	adult	Kahikatea Swamp Forest Walk, Haast	Brunner/Westland
E1080	040510.18	*B. bassiana*	Coleoptera: Scarabaeidae	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman
E1073	040513.6	*B. bassiana*	*Stethaspsis suturalis* (Coleoptera: Scarabaeidae)	adult	Rolling Creek, Wangapeka Valley	Nelson/Tasman
E94	030422.2	*B. caledonica*	Coleoptera	adult	Terrace Walk, Franz Josef	Brunner/Westland
NC49	050406.4	*B. caledonica*	Coleoptera	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC142	060415.3	*B. caledonica*	*Prionoplus reticularis* (Coleoptera: Cerambycidae)	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
NC95	050418.2	*B. caledonica*	Coleoptera: Curculionidae	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
E222	030509.18	*B. caledonica*	*Stethaspsis longicornis* (Coleoptera: Scarabaeidae)	adult	Aongate Short Loop Track, Katikati	Bay of Plenty
E205	030509.8	*B. malawiensis*	*Cicindela* sp. (Coleoptera: Carabidae)	larva	Aongatete Short Loop Track, Katikati	Bay of Plenty
E220	030509.7	*B. malawiensis*	*Cicindela* sp.	larva	Aongatete Short Loop Track, Katikati	Bay of Plenty
E195	030506.1	*B. malawiensis*	*Cicindela* sp.	larva	Lindemann Loop Track, Katikati	Bay of Plenty
E196	030506.4	*B. malawiensis*	*Cicindela* sp.	larva	Lindemann Loop Track, Katikati	Bay of Plenty

Table 3.7 continued.

Isolate #	Specimen #	Species	Host	Host stage	Locality	Region
NC214	060511.3	*B. malawiensis*	Coleoptera: Curculionidae	adult	Nile River Valley Walk, Charleston	Brunner/Westland
E188	03 0506.12	*B. malawiensis*	*Pyronota festiva* (Coleoptera: Scarabaeidae)	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
NC202	060508.3	*B. malawiensis*	Coleoptera: Scarabaeidae	adult	Charming Creek Walkway, Westport	Nelson/Tasman
NC188	060415.51	*B. malawiensis*	Coleoptera: Scarabaeidae	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
E1079	040513.17	*B. caledonica*	Dermaptera	adult	Rolling Creek, Wangapeka Valley	Nelson/Tasman
NC225	060510.1	*B.brongniartii*	*Procyliosoma tuberculatum* (Diplopoda: Sphaerotheriidae)	adult	Oparara Arch Walk, Karamea	Nelson/Tasman
T875*	n.a.	*B. bassiana*	*Arachnocampa luminosa* (Diptera: Keroplatidae)	adult	Waitomo Caves	Waikato
NC123	050408.22	*B. bassiana*	Hemiptera: Cicadidae	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC208	060509.6	*B. bassiana*	*Amphipsalta. zealandica* (Hemiptera: Cicadidae)	adult	Nikau Loop Walk, Karamea	Nelson/Tasman
NC209	060509.7	*B. bassiana*	*A. zealandica*	adult	Nikau Loop Walk, Karamea	Nelson/Tasman
NC228	060509.1	*B. bassiana*	*A. zealandica*	adult	Nikau Loop Walk, Karamea	Nelson/Tasman
TE439	020417.6	*B. bassiana*	*A. zealandica*	adult	Goldsborough (Shamrock)Track, Hokitika	Brunner/Westland
NC111	050404.11	*B. bassiana*	*Amphipsalta* sp.	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC48	050408.2	*B. caledonica*	Hemiptera: Cicadidae	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC168	060415.27	*B. malawiensis*	Hemiptera	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
E190	030509.34	*B. malawiensis*	Hemiptera: Cicadidae	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E219	030507.1	*B. malawiensis*	Hemiptera: Cicadidae	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E96	030422.6	*B. malawiensis*	Hemiptera: Cicadidae	adult	Terrace Walk, Franz Josef	Brunner/Westland
TE672	020507.2	*B. malawiensis*	Hemiptera: Cicadidae	adult	Hapuka Estuary Walk, Okuru	Brunner/Westland
NC205	060508.1	*B. malawiensis*	*A. zealandica*	adult	Nikau Loop Walk, Karamea	Nelson/Tasman
NC78	050315.16	*B. malawiensis*	*A. zealandica*	adult	Truman Track, Punakaiki	Brunner/Westland
E221	030509.15	*B. malawiensis*	*Monteithiella humeralis* (Hemiptera: Pentatomidae)	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty

Table 3.7 continued.

Isolate #	Specimen #	Species	Host	Host stage	Locality	Region
E1063	040513.3	*B. bassiana*	*Vespula germanica* (Hymenoptera: Vespidae)	adult	Rolling Creek, Wangapeka Valley	Nelson/Tasman
E1069	040511.12	*B. bassiana*	*V. germanica*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1068	040511.27	*B. bassiana*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1070	040511.17	*B. bassiana*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1175	040511.24	*B. bassiana*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1057	040514.9	*B. bassiana*	*Vespula vulgaris*	adult	Loop Track, Lake Rotoiti	Nelson/Tasman
E1064	040514.6	*B. bassiana*	*Vespula* sp.	adult	Loop Track, Lake Rotoiti	Nelson/Tasman
E1065	040510.10	*B. bassiana*	*Vespula* sp.	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman
E1067	040513.15	*B. bassiana*	*Vespula* sp.	adult	Rolling Creek, Wangapeka Valley	Nelson/Tasman
T884	000408.1	*B. malawiensis*	*Certonotus fractinervis* (Hymenoptera: Ichneumonidae)	adult	Mount French Track, Lake Brunner	Brunner/Westland
E197	030506.13	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E201	030509.4	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E206	030509.12	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E207	030509.14	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E208	030509.16	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E210	030509.26	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E215	030509.35	*B. malawiensis*	*V. vulgaris*	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E1059	040511.18	*B. malawiensis*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1060	040511.20	*B. malawiensis*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1066	040511.19	*B. malawiensis*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
E1084	040510.10	*B. malawiensis*	*V. vulgaris*	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman
E1176	040511.38	*B. malawiensis*	*V. vulgaris*	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
NC210	060509.8	*B. malawiensis*	*V. vulgaris*	adult	Nikau Loop Walk, Karamea	Nelson/Tasman
NC215	060511.4	*B. malawiensis*	*V. vulgaris*	adult	Nile River Valley Walk, Charleston	Brunner/Westland

Table 3.7 continued.

Isolate #	Specimen #	Species	Host	Host stage	Locality	Region
E202	030509.5	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E203	030509.6	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E211	030509.27	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E213	030509.29	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E214	030509.30	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
E216	030509.2	*B. malawiensis*	*Vespula* sp.	adult	Aongatete Short Loop Track, Katikati	Bay of Plenty
T885	000408.5	*B. malawiensis*	*Vespula* sp.	adult	Mount French Track, Lake Brunner	Nelson/Tasman
NC222	060512.5	*B. malawiensis*	*Vespula* sp.	adult	Oparara Arch Walk, Karamea	Brunner/Westland
NC107	050408.11	*B. bassiana*	Orthoptera	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
E1139	040510.1	*B. bassiana*	*Phaulacridium marginale* (Orthoptera: Acrididae)	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman
NC219	0605012.1	*B. bassiana*	Orthoptera: Tettigoniidae	adult	Truman Track, Punakaiki	Brunner/Westland
NC83	050412.1	*B. malawiensis*	*P. marginale*	adult	Lake Kaniere Walkway, Hokitika	Brunner/Westland
NC220	060512.2	*B. malawiensis*	Orthoptera: Tettigoniidae	adult	Truman Track, Punakaiki	Brunner/Westland
NC43	050405.8	*B. bassiana*	Phasmatodea: Phasmatidae	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
TE445	n.a.	*B. malawiensis*	*Clitarchus hookeri* (Phasmatodea: Phasmatidae)	adult	Hapuka Estuary Walk, Okuru	Brunner/Westland
NC96	050510.8	*B. bassiana*	n.d	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
NC99	050418.1	*B. bassiana*	n.d.	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
NC100	050510.1	*B. bassiana*	n.d.	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
NC101	050418.5	*B. bassiana*	n.d	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
NC104	050418.6	*B. bassiana*	n.d.	adult	Devils Punchbowl Track, Arthurs Pass	North Canterbury
NC82	050320.2	*B. bassiana*	n.d.	adult	Wooded Gully Track, Mount Thomas	North Canterbury
NC88	050320.10	*B. bassiana*	n.d.	adult	Wooded Gully Track, Mount Thomas	North Canterbury
NC92	050320.14	*B. bassiana*	n.d.	adult	Wooded Gully Track, Mount Thomas	North Canterbury
E1083	040510.15	*B. bassiana*	n.d.	adult	Snowdens Bush Scenic Reserve, Brightwater	Nelson/Tasman

Table 3.7 continued.

Isolate #	Specimen #	Species	Host	Host stage	Locality	Region
E1174	040511.22	*B. bassiana*	n.d.	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman
NC41	050408.1	*B. bassiana*	n.d.	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC110	050408.5	*B. bassiana*	n.d	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC119	050408.4	*B. bassiana*	n.d.	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
NC44	050408.12	*B. caledonica*	n.d.	adult	Mangawhero Forest Walk, Mount Ruapehu	Tongariro/Rangitikei
E1077	040511.15	*B. malawiensis*	n.d.	adult	Eves Valley Scenic Reserve, Brightwater	Nelson/Tasman

n.a., not available; n.d., not determined; * culture provided by N. Waipara, AgResearch, Ruakura, February 2000.

3.3.2 Morphological characterisation

Beauveria bassiana

Conidia from *B. bassiana* specimens were globose to subglobose (Fig. 3.2A) and measured 1.6-2.6 × 1.3-2.4 µm (average 2.0 × 1.8 µm) on the host. Conidia from cultures on ¼ SDYA after 14 days measured 1.8-3.0 × 1.6-2.8 µm (average 2.3 × 2.0 µm). Conidial sizes from different host orders are shown in Table 3.8. There was no significant correlation between conidial size and host order.

Table 3.8 Conidial sizes of *B. bassiana* from different host orders. Measurements are given in µm with average values in brackets.

Host order	Conidia on host length × width	Conidia on ¼SDYA length × width
Aranaea	1.9-2.3 (2.0) × 1.7-2.1 (1.9)	2.0-2.9 (2.5) × 1.8-2.5 (2.1)
Blattodea	1.6-2.5 (2.0) × 1.6-2.2 (1.9)	1.9-2.6 (2.2) × 1.6-2.3 (2.0)
Coleoptera	1.6-2.6 (2.0) × 1.4-2.4 (1.8)	1.9-2.9 (2.3) × 1.7-2.7 (2.0)
Hemiptera	1.6-2.4 (2.0) × 1.4-2.2 (1.8)	1.9-3.0 (2.3) × 1.7-2.7 (2.1)
Hymenoptera	1.7-2.5 (2.0) × 1.3-2.3 (1.8)	1.9-2.8 (2.3) × 1.7-2.6 (2.1)
Orthoptera	1.7-2.3 (2.0) × 1.4-2.1 (1.8)	1.9-2.9 (2.4) × 1.7-2.6 (2.2)
Phasmatodea	1.6-2.1 (1.9) × 1.5-2.0 (1.7)	1.9-2.5 (2.2) × 1.7-2.5 (2.0)
Unidentified	1.6-2.3 (2.0) × 1.3-2.2 (1.7)	1.8-2.9 (2.3) × 1.6-2.8 (2.0)

PDD26260, PDD35494, PDD35523 (received as *B. densa*); PDD73899 and PDD34800 (received as *B. brongniartii*) were all identified as *B. bassiana* with globose to subglobose conidia ranging from 1.6-3.0 × 1.4-2.6 µm. Another specimen labelled *B. densa* (PDD13960) had ellipsoidal to fusiform, catenate conidia and whorls of ellipsoidal to cylindrical phialides and was identified as *Isaria farinosa.*

Beauveria brongniartii

Isolate NC225 was initially identified as *B. bassiana* with globose to subglobose conidia (Fig. 3.2E) measuring 2.0-2.6 × 1.8-2.2 µm (average 2.3 × 1.9 µm) on the host and 2.1-3.0 × 1.9-2.7 µm (average 2.6 × 2.2 µm) on ¼ SDYA after 14 days. However, phylogenetic analyses (Figures 3.7-3.10) identified the isolate as *B. brongniartii.* PDD25211 (dried culture, received as *B. tenella*) had subglobose to ellipsoidal conidia measuring 2.0-3.7 × 2.0 -3.3 µm (average 3.1 × 2.5 µm) and was identified as *B. brongniartii.*

Beauveria malawiensis

Conidia from *B. malawiensis* specimens were cylindrical (Fig. 3.4A) and measured 2.9-4.1 × 1.1-1.9 µm (average 3.4 × 1.4 µm) on the host. Conidia from cultures on ¼ SDYA after 14 days measured 3.0-4.8 × 1.1-2.0 µm (average 3.5 × 1.5 µm). Conidial sizes from different host orders are shown in Table 3.9. There was no significant correlation between conidial size and host order.

Table 3.9 Conidial sizes of *B. malawiensis* from different host orders. Measurements are given in µm with average values in brackets.

Host order	Conidia on host length × width	Conidia on ¼ SDYA length × width
Coleoptera	3.0-4.1 (3.4) × 1.2-1.8 (1.4)	3.0-4.8 (3.6) × 1.2-2.0 (1.5)
Hemiptera	3.0-4.1 (3.4) × 1.1-1.8 (1.5)	3.0-4.3 (3.6) × 1.2-2.0 (1.5)
Hymenoptera	2.9-4.1 (3.4) × 1.1-1.9 (1.4)	3.0-4.4 (3.5) × 1.2-2.0 (1.5)
Orthoptera	3.0-3.8 (3.3) × 1.2-1.8 (1.4)	3.1-4.2 (3.6) × 1.3-1.7 (1.5)
Phasmatodea	not available	3.3-4.2 (3.7) × 1.2-1.7 (1.5)
Unidentified	3.0-4.0 (3.4) × 1.2-1.5 (1.4)	3.0-4.1 (3.6) × 1.1-1.7 (1.4)

Excluding PDD34800 and PDD73899, all specimens from PDD received as *B. brongniartii* were identified as *B. malawiensis* with cylindrical conidia measuring from 3.0-4.0 × 1.1-2.0 µm.

Beauveria caledonica

Conidia from *B. caledonica* were ellipsoidal to cylindrical and often slightly curved (Fig. 3.6A-B). Conidia measured 2.2-3.3 × 1.1-1.6 µm (average 2.7 × 1.4 µm) on the host. Conidia from cultures on ¼ SDYA after 14 days measured 2.8-5.1 × 1.0-2.1 µm (average 3.5 × 1.4 µm). Conidial sizes from different host orders are shown in Table 3.10. There was no significant correlation between conidial size and host order.

Table 3.10 Conidial sizes of *B. caledonica* from different host orders. Measurements are given in µm with average values in brackets.

Host order	Conidia on host length × width	Conidia on ¼ SDYA length × width
Coleoptera	2.2-3.3 (2.7) × 1.1-1.6 (1.4)	2.9-4.7 (3.5) × 1.0-2.1 (1.4)
Dermaptera	2.5-3.1 (2.8) × 1.2-1.6 (1.3)	3.0-5.1 (3.4) × 1.1-1.7 (1.3)
Hemiptera	2.4-3.1 (2.8) × 1.2-1.6 (1.4)	3.0-4.4 (3.7) × 1.2-1.7 (1.5)
Unidentified	2.4-3.1 (2.7) × 1.1-1.4 (1.2)	2.8-4.3 (3.3) × 1.1-1.7 (1.4)

Table 3.11 Measurements of conidia from *Beauveria* specimens and cultures. All measurements are in μm with averages (n=25) in brackets.

Isolate #	Specimen #	Species	Host	Region	Conidia on ¼ SDYA length × width	Conidia on host length × width
NC87	050320.11	*B. bassiana*	Araneae	North Canterbury	2.0-2.9 (2.5) × 1.8-2.5 (2.1)	1.9-2.3 (2.0) × 1.7-2.1 (1.9)
NC79	050215.2	*B. bassiana*	Blattodea	North Canterbury	1.9-2.4 (2.1) × 1.6-2.2 (1.9)	1.6-2.2 (2.0) × 1.6-2.1 (1.8)
NC84	050412.2	*B. bassiana*	Blattodea	Brunner/Westland	1.9-2.6 (2.2) × 1.6-2.3 (2.0)	1.8-2.5 (2.1) × 1.6-2.2 (1.9)
NC85	050412.3	*B. bassiana*	Coleoptera	Brunner/Westland	2.0-2.7 (2.3) × 1.8-2.4 (2.0)	1.7-2.2 (2.0) × 1.5-2.2 (1.8)
NC86	050412.4	*B. bassiana*	Coleoptera	Brunner/Westland	2.0-2.5 (2.3) × 1.8-2.4 (2.0)	1.9-2.3 (2.1) × 1.7-2.2 (2.0)
NC97	050301.1	*B. bassiana*	Coleoptera	Brunner/Westland	2.0-2.6 (2.2) × 1.7-2.4 (2.0)	1.8-2.2 (1.9) × 1.5-2.1 (1.8)
NC53	050408.6	*B. bassiana*	Coleoptera	Tongariro/Rangitikei	2.0-2.5 (2.2) × 1.7-2.3 (2.0)	1.6-2.0 (1.8) × 1.4-1.9(1.6)
NC62	050406.31	*B. bassiana*	Coleoptera	Tongariro/Rangitikei	1.9-2.6 (2.2) × 1.8-2.3 (2.0)	1.7-2.0 (1.8) × 1.5-1.8 (1.6)
E1082	040510.17	*B. bassiana*	Coleoptera	Nelson/Tasman	1.9-2.4 (2.2) × 1.7-2.2 (1.9)	1.9-2.3 (2.1) × 1.6-2.1 (1.8)
NC106	050406.34	*B. bassiana*	Coleoptera	Tongariro/Rangitikei	2.0-2.9 (2.5) × 1.8-2.5 (2.1)	1.7-2.3 (1.9) × 1.4-1.9 (1.6)
TE833	020506.1	*B. bassiana*	Coleoptera	Brunner/Westland	2.2-2.8 (2.4) × 1.8-2.7 (2.1)	2.0-2.6 (2.2) × 1.8-2.4 (2.0)
E1080	040510.18	*B. bassiana*	Coleoptera	Nelson/Tasman	2.1-2.9 (2.3) × 1.8-2.3 (2.0)	2.0-2.5 (2.3) × 1.8-2.2 (2.0)
E1073	040513.6	*B. bassiana*	Coleoptera	Nelson/Tasman	1.9-2.6 (2.2) × 1.7-2.4 (1.9)	1.9-2.5 (2.2) × 1.8-2.3 (2.1)
E94	030422.2	*B. caledonica*	Coleoptera	Brunner/Westland	3.0-4.7 (3.8) × 1.2-2.1 (1.7)	2.5-3.3 (2.8) × 1.2-1.6 (1.4)
NC49	050406.4	*B. caledonica*	Coleoptera	Tongariro/Rangitikei	2.9-4.4 (3.5) × 1.0-1.8 (1.3)	2.3-3.1 (2.7) × 1.1-1.6 (1.4)
NC142	060415.3	*B. caledonica*	Coleoptera	Brunner/Westland	2.9-4.3 (3.3) × 1.1-1.6 (1.4)	2.2.2-9 (2.5) × 1.1-1.6 (1.4)
NC95	050418.2	*B. caledonica*	Coleoptera	North Canterbury	2.9-4.5 (3.4) × 1.2-1.6 (1.3)	2.3-3.1 (2.7) × 1.1-1.5 (1.3)
E222	030509.18	*B. caledonica*	Coleoptera	Bay of Plenty	2.9-4.3 (3.3) × 1.2-1.9 (1.5)	2.4-3.1 (2.7) × 1.2-1.6 (1.4)
E205	030509.8	*B. malawiensis*	Coleoptera	Bay of Plenty	3.1-4.1 (3.6) × 1.3-2.0 (1.6)	3.0-4.1 (3.5) × 1.2-1.8 (1.4)
E220	030509.7	*B. malawiensis*	Coleoptera	Bay of Plenty	3.1-3.9 (3.5) × 1.3-2.0 (1.6)	3.0-3.8 (3.4) × 1.3-1.6 (1.5)
E195	030506.1	*B. malawiensis*	Coleoptera	Bay of Plenty	3.1-4.0 (3.5) × 1.2-1.8 (1.5)	3.2-4.0 (3.5) × 1.2-1.6 (1.4)
E196	030506.4	*B. malawiensis*	Coleoptera	Bay of Plenty	3.0-4.0 (3.5) × 1.2-1.7 (1.3)	3.0-3.7 (3.3) × 1.2-1.8 (1.5)
NC214	060511.3	*B. malawiensis*	Coleoptera	Brunner/Westland	3.4-4.8 (4.0) × 1.3-1.8 (1.5)	3.0-3.8 (3.4) × 1.2-1.6 (1.5)
E188	030506.12	*B. malawiensis*	Coleoptera	Bay of Plenty	3.1-4.2 (3.6) × 1.3-1.8 (1.6)	3.0-4.0 (3.4) × 1.3-1.6 (1.5)
NC202	060508.3	*B. malawiensis*	Coleoptera	Nelson/Tasman	3.2-3.9 (3.5) × 1.3-1.7 (1.4)	3.3-3.8 (3.6) × 1.2-1.6 (1.3)

Table 3.11 continued.

Isolate #	Specimen #	Species	Host	Region	Conidia on ¼ SDYA length × width	Conidia on host length × width
NC188	060415.51	*B. malawiensis*	Coleoptera	Brunner/Westland	3.1-4.0 (3.6) × 1.2-1.9 (1.4)	3.0-3.8 (3.3) × 1.2-1.6 (1.3)
E1079	040513.17	*B. caledonica*	Dermaptera	Nelson/Tasman	3.0-5.1 (3.4) × 1.1-1.7 (1.3)	2.5-3.1 (2.8) × 1.2-1.6 (1.3)
NC225	060510.1	*B. bassiana*	Diplopoda	Nelson/Tasman	2.1-2.8 (2.4) × 1.8-2.2 (2.0)	2.0-2.6 (2.3) × 1.8-2.2 (1.9)
T875	n.a.	*B. bassiana*	Diptera	Waikato	2.1-2.5 (2.3) × 1.9-2.4 (2.1)	n.a.
NC123	050408.22	*B. bassiana*	Hemiptera	Tongariro/Rangitikei	2.1-3.0 (2.6) × 1.9-2.7 (2.2)	1.7-2.4 (2.1) × 1.6-2.2 (1.9)
NC208	060509.6	*B. bassiana*	Hemiptera	Nelson/Tasman	2.0-2.5 (2.3) ×1.8-2.3 (2.1)	1.8-2.3 (2.0) × 1.5-2.1 (1.8)
NC209	060509.7	*B. bassiana*	Hemiptera	Nelson/Tasman	1.9-2.6 (2.2) × 1.9-2.3 (2.1)	1.7-2.1 (1.9) × 1.4-1.9 (1.7)
NC228	060509.1	*B. bassiana*	Hemiptera	Nelson/Tasman	1.9-2.4 (2.2) × 1.7-2.2 (2.0)	1.8-2.3 (2.1) × 1.7-2.1 (1.9)
TE439	020417.6	*B. bassiana*	Hemiptera	Brunner/Westland	1.9-2.4 (2.2) × 1.8-2.1 (1.9)	1.9-2.4 (2.2) × 1.8-2.1 (2.0)
NC111	050404.11	*B. bassiana*	Hemiptera	Tongariro/Rangitikei	2.0-2.7 (2.3) × 1.8-2.5 (2.1)	1.6-2.0 (1.8) × 1.4-2.0 (1.7)
NC48	050408.2	*B. caledonica*	Hemiptera	Tongariro/Rangitikei	3.0-4.4 (3.7) × 1.2-1.7 (1.5)	2.4-3.1 (2.8) × 1.2-1.6 (1.4)
NC168	060415.27	*B. malawiensis*	Hemiptera	Brunner/Westland	3.2-4.2 (3.6) × 1.3-1.9 (1.5)	3.0-4.0 (3.4) × 1.2-1.6 (1.4)
E190	030509.34	*B. malawiensis*	Hemiptera	Bay of Plenty	3.0-3.9 (3.4) × 1.2-1.8 (1.5)	3.0-3.8 (3.3) × 1.2-1.5 (1.3)
E219	030507.1	*B. malawiensis*	Hemiptera	Bay of Plenty	3.2-4.2 (3.6) × 1.3-1.9 (1.5)	3.0-3.7 (3.3) × 1.3-1.6 (1.5)
E96	030422.6	*B. malawiensis*	Hemiptera	Brunner/Westland	3.0-3.8 (3.4) × 1.2-1.7 (1.5)	3.1-3.7 (3.4) × 1.1-1.7 (1.4)
TE672	020507.2	*B. malawiensis*	Hemiptera	Brunner/Westland	3.2-4.0 (3.6) × 1.2-1.8 (1.5)	3.0-3.6 (3.4) × 1.4-1.8 (1.6)
NC205	060508.1	*B. malawiensis*	Hemiptera	Nelson/Tasman	3.3-4.3 (3.6) × 1.2-1.7 (1.4)	3.0-3.9 (3.5) × 1.2-1.8 (1.5)
NC78	050315.16	*B. malawiensis*	Hemiptera	Brunner/Westland	3.2-4.2 (3.7) × 1.3-1.6 (1.4)	3.0-4.1 (3.5) × 1.2-1.7 (1.4)
E221	030509.15	*B. malawiensis*	Hemiptera	Bay of Plenty	3.2-4.2 (3.7) × 1.5-2.0 (1.7)	3.0-3.8 (3.4) × 1.2-1.6 (1.4)
E1063	040513.3	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.1-2.7 (2.5) × 1.9-2.6 (2.2)	1.9-2.5 (2.1) × 1.7-2.0 (1.9)
E1069	040511.12	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.1-2.7 (2.4) × 1.8-2.6 (2.1)	1.9-2.3 (2.1) × 1.6-2.3 (1.8)
E1068	040511.27	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.1-2.8 (2.4) × 1.8-2.6 (2.2)	1.7-2.3 (2.1) × 1.6-2.2 (1.9)
E1070	040511.17	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.0-2.4 (2.2) × 1.7-2.3 (1.9)	1.7-2.0 (1.8) × 1.3-1.8 (1.6)
E1175	040511.24	*B. bassiana*	Hymenoptera	Nelson/Tasman	1.9-2.3 (2.1) × 1.8-2.2 (1.9)	1.9-2.3 (2.1) × 1.7-2.2 (2.0)
E1057	040514.9	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.0-2.6 (2.3) × 1.7-2.6 (2.1)	1.7-2.2 (1.9) × 1.5-2.0 (1.7)

Table 3.11 continued.

Isolate #	Specimen #	Species	Host	Region	Conidia on ¼ SDYA length × width	Conidia on host length × width
E1064	040514.6	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.0-2.8 (2.3) × 1.7-2.6 (2.0)	1.9-2.5 (2.1) × 1.7-2.2 (1.9)
E1065	040510.10	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.0-2.8 (2.3) × 1.7-2.5 (2.0)	1.7-2.4 (2.0) × 1.5-2.2 (1.8)
E1067	040513.15	*B. bassiana*	Hymenoptera	Nelson/Tasman	2.2-2.8 (2.4) × 1.7-2.6 (2.1)	1.7-2.3 (2.0) × 1.5-2.2 (1.8)
T884	000408.1	*B. malawiensis*	Hymenoptera	Brunner/Westland	3.1-4.2 (3.7) × 1.3-1.9 (1.5)	3.0-3.7 (3.4) × 1.3-1.6 (1.5)
E197	030506.13	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.2-4.0 (3.6) × 1.2-1.6 (1.4)	2.9-3.5 (3.2) × 1.1-1.5 (1.3)
E201	030509.4	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.2-4.2 (3.5) × 1.3-1.8 (1.5)	2.9-3.5 (3.2) × 1.3-1.6 (1.4)
E206	030509.12	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-3.8 (3.3) × 1.2-1.8 (1.5)	3.0-3.7 (3.3) × 1.2-1.6 (1.3)
E207	030509.14	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-3.9 (3.4) × 1.3-1.8 (1.4)	3.0-3.8 (3.4) × 1.2-1.6 (1.4)
E208	030509.16	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.2-4.1 (3.7) × 1.2-1.9 (1.6)	3.0-4.0 (3.5) × 1.2-1.6 (1.4)
E210	030509.26	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-3.6 (3.2) × 1 .2-1.8 (1.4)	3.0-3.6 (3.3) × 1.2-1.6 (1.4)
E215	030509.35	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.1-4.3 (3.3) × 1.2-1.7 (1.4)	2.9-3.7 (3.4) × 1.2-1.8 (1.5)
E1059	040511.18	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.1-4.4 (3.6) × 1.3-2.0 (1.5)	3.0-3.8 (3.3) × 1.1-1.6 (1.4)
E1060	040511.20	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.0-4.3 (3.5) × 1.3-1.8 (1.5)	3.1-3.9 (3.5) × 1.2-1.7 (1.4)
E1066	040511.19	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.2-4.2 (3.7) × 1.2-1.8 (1.5)	3.0-3.8 (3.4) × 1.1-1.5 (1.3)
E1084	040510.10	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.0-4.0 (3.3) × 1.1-1.7 (1.4)	2.9-3.4 (3.1) × 1.2-1.6 (1.4)
E1176	040511.38	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.1-4.2 (3.6) × 1.3-1.8 (1.6)	3.1-3.9 (3.5) × 1.2-1.7 (1.4)
NC210	060509.8	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.2-4.2 (3.5) × 1.2-1.7 (1.4)	2.9-3.7(3.2) × 1.3-1.7 (1.5)
NC215	060511.4	*B. malawiensis*	Hymenoptera	Brunner/Westland	3.1-4.3 (3.6) × 1.2-1.7 (1.4)	3.0-3.7 (3.4) × 1.3-1.8 (1.5)
E202	030509.5	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-4.1 (3.5) × 1.2-1.7 (1.4)	3.1-3.7 (3.3) × 1.2-1.5 (1.3)
E203	030509.6	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.1-4.0 (3.5) × 1.2-1.7 (1.5)	3.1-4.0 (3.6) × 1.2-1.6 (1.3)
E211	030509.27	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-4.1 (3.4) × 1.2-1.6 (1.4)	3.0-3.6 (3.4) × 1.2-1.6 (1.4)
E213	030509.29	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.2-4.0 (3.5) × 1.2-1.7 (1.5)	3.0-3.5 (3.2) × 1.2-1.7 (1.5)
E214	030509.30	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.0-4.0 (3.5) × 1.2-1.8 (1.4)	2.9-3.4 (3.2) × 1.1-1.6 (1.3)
E216	030509.2	*B. malawiensis*	Hymenoptera	Bay of Plenty	3.1-4.2 (3.5) × 1.3-1.8 (1.6)	2.9-3.8 (3.3) × 1.1-1.8 (1.4)
T885	000408.5	*B. malawiensis*	Hymenoptera	Brunner/Westland	3.1-4.1 (3.6) × 1.2-1.7 (1.4)	3.1-4.1 (3.6) × 1.3-1.7 (1.6)

Table 3.11 continued.

Isolate #	Specimen #	Species	Host	Region	Conidia on ¼ SDYA length × width	Conidia on host length × width
NC222	060512.5	*B. malawiensis*	Hymenoptera	Nelson/Tasman	3.1-4.3 (3.7) × 1.3-2.0 (1.7)	3.2-4.0 (3.6) × 1.2 1.7 (1.5)
NC107	050408.11	*B. bassiana*	Orthoptera	Tongariro/Rangitikei	1.9-2.6 (2.3) × 1.7-2.2 (2.0)	1.7-2.1 (1.9) × 1.4-1.9 (1.6)
E1139	040510.1	*B. bassiana*	Orthoptera	Nelson/Tasman	1.9-2.8 (2.3) × 1.7-2.4 (2.1)	1.8-2.3 (2.1) × 1.6-2.1 (1.9)
NC219	0605012.1	*B. bassiana*	Orthoptera	Brunner/Westland	2.2-2.9 (2.6) × 2.0-2.6 (2.4)	1.7-2.3 (2.1) × 1.5-1.9 (1.8)
NC83	050412.1	*B. malawiensis*	Orthoptera	Brunner/Westland	3.1-4.2 (3.5) × 1.3-1.7 (1.5)	3.0-3.8 (3.2) × 1.2-1.5 (1.4)
NC220	060512.2	*B. malawiensis*	Orthoptera	Brunner/Westland	3.3-4.2 (3.7) × 1.3-1.7 (1.5)	3.0-3.7 (3.3) × 1.2-1.8 (1.4)
NC43	050405.8	*B. bassiana*	Phasmatodea	Tongariro/Rangitikei	1.9-2.5 (2.2) × 1.7-2.5 (2.0)	1.6-2.1 (1.9) × 1.5-2.0 (1.7)
TE445	n.a.	*B. malawiensis*	Phasmatodea	Brunner/Westland	3.3-4.2 (3.7) × 1.2-1.7 (1.5)	n.a.
NC96	050510.8	*B. bassiana*	n.d	North Canterbury	1.9-2.7 (2.2) × 1.7-2.5 (2.0)	1.9-2.3 (2.1) × 1.7-2.2 (1.9)
NC99	050418.1	*B. bassiana*	n.d.	North Canterbury	2.0-2.6 (2.4) × 1.8-2.3 (2.1)	1.7-2.0 (1.9) × 1.4-1.8 (1.5)
NC100	050510.1	*B. bassiana*	n.d.	North Canterbury	1.9-2.6 (2.2) × 1.7-2.6 (2.0)	1.8-2.2 (2.1) × 1.6-2.2 (1.9)
NC101	050418.5	*B. bassiana*	n.d	North Canterbury	2.1-2.9 (2.5) × 1.8-2.8 (2.2)	1.6-2.1 (1.8) × 1.4-2.0 (1.6)
NC104	050418.6	*B. bassiana*	n.d.	North Canterbury	2.0-2.7 (2.3) × 1.6-2.4 (1.9)	1.8-2.2 (2.0) × 1.5-1.9 (1.7)
NC82	050320.2	*B. bassiana*	n.d.	North Canterbury	1.8-2.3 (2.0) × 1.6-2.3 (1.9)	1.7-2.1 (2.0) × 1.5-1.9 (1.7)
NC88	050320.10	*B. bassiana*	n.d.	North Canterbury	1.8-2.5 (2.1) × 1.6-2.1 (1.9)	1.7-2.2 (1.9) × 1.5-1.9 (1.7)
NC92	050320.14	*B. bassiana*	n.d.	North Canterbury	2.0-2.7 (2.3) × 1.9-2.5 (2.1)	1.8-2.1 (1.9) × 1.3-2.0 (1.7)
E1083	040510.15	*B. bassiana*	n.d.	Nelson/Tasman	2.2-2.9 (2.5) × 2.0-2.6 (2.3)	1.9-2.3 (2.1) × 1.6-2.0 (1.8)
E1174	040511.22	*B. bassiana*	n.d.	Nelson/Tasman	2.0-2.7 (2.3) × 1.8-2.5 (2.0)	1.6-2.1 (1.9) × 1.5-2.0 (1.6)
NC41	050408.1	*B. bassiana*	n.d.	Tongariro/Rangitikei	2.0-2.6 (2.2) × 1.8-2.5 (2.1)	1.7-2.2 (2.0) × 1.6-2.2 (1.8)
NC110	050408.5	*B. bassiana*	n.d	Tongariro/Rangitikei	2.0-2.9 (2.3) × 1.8-2.6 (2.1)	1.7-2.1 (1.9) × 1.5-2.0 (1.7)
NC119	050408.4	*B. bassiana*	n.d.	Tongariro/Rangitikei	1.9-2.9 (2.4) × 1.8-2.6 (2.1)	1.7-2.2 (2.0) × 1.6-2.1 (1.7)
NC44	050408.12	*B. caledonica*	n.d.	Tongariro/Rangitikei	2.8-4.3 (3.3) × 1.1-1.7 (1.4)	2.4-3.1 (2.7) × 1.1-1.4 (1.2)
E1077	040511.15	*B. malawiensis*	n.d.	Nelson/Tasman	3.0-4.1 (3.6) × 1.1-1.7 (1.4)	3.0-4.0 (3.4) × 1.2-1.5 (1.4)

n.a., not available; n.d., not determined

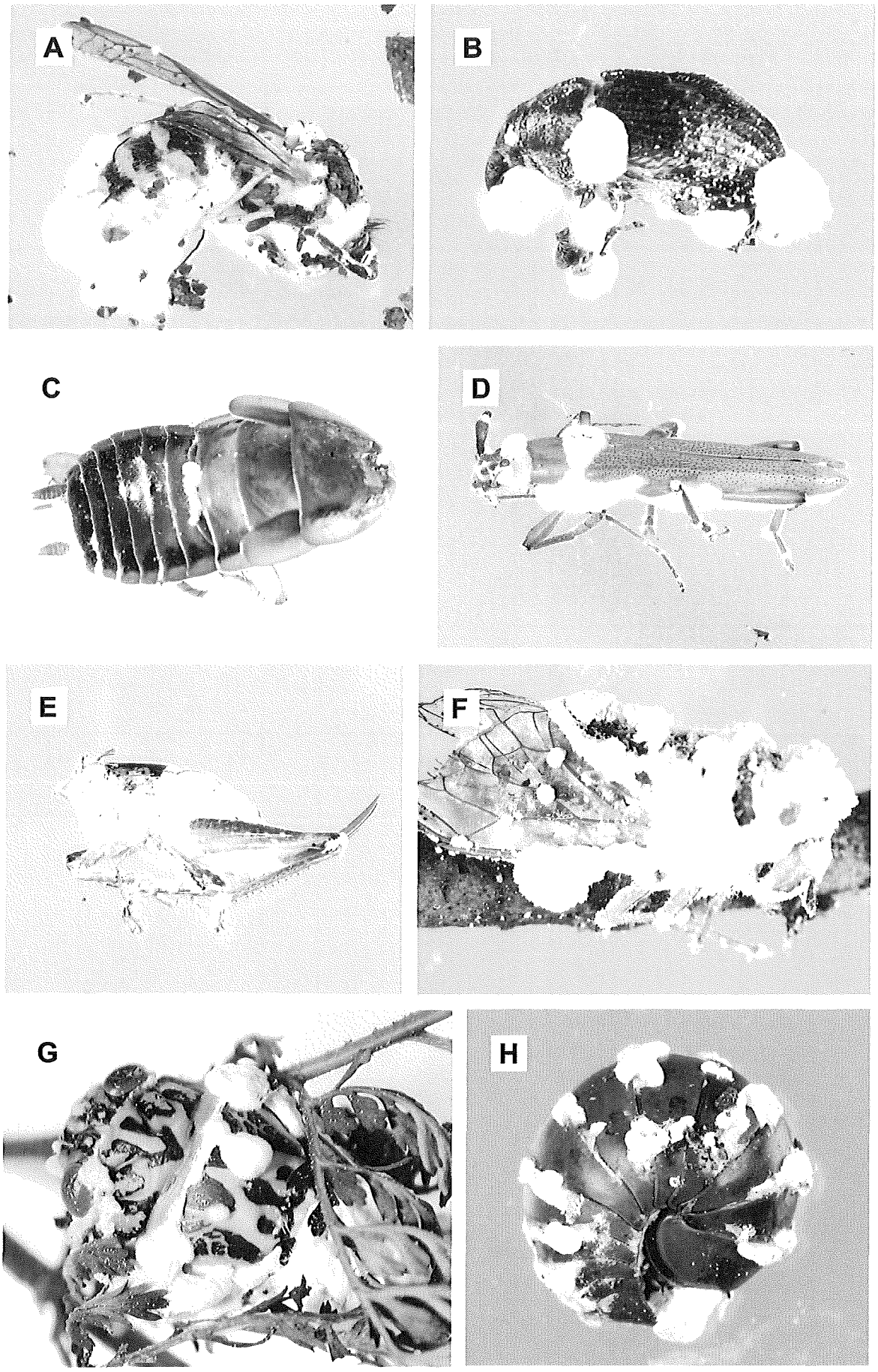


Figure 3.1 A-G *Beauveria bassiana* on various hosts: **A** Hymenoptera, 040511.27; **B** Coleoptera, 050406.34; **C** Blattodea, 050215.2; **D** Coleoptera, 040510.1; **E** Orthoptera, 040510.1; **F** Hemiptera, 050408.2; **G** Hemiptera, 020417.6. **H** *Beauveria brongniartii* on Sphaerotheriida, 060510.1.

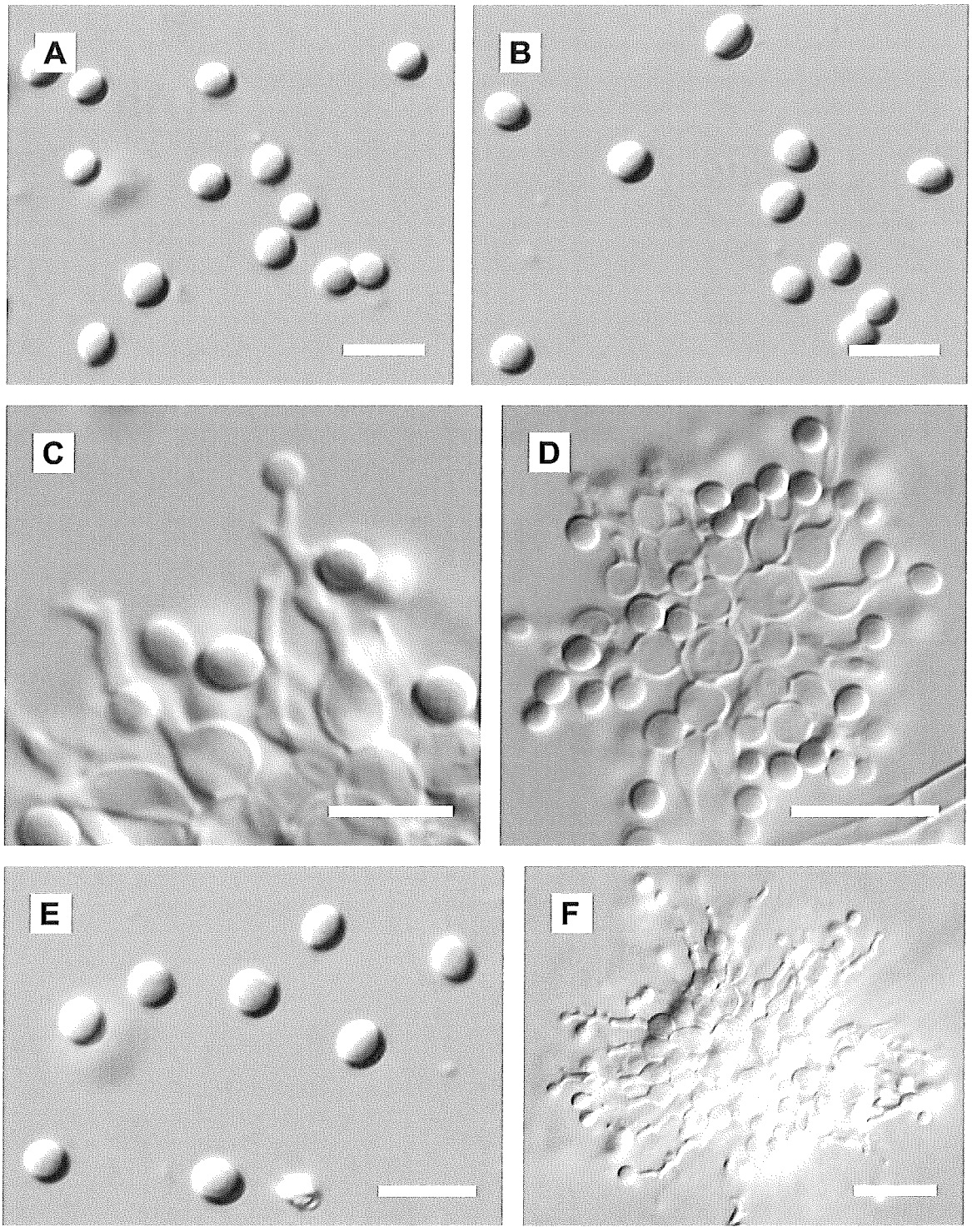


Figure 3.2. A-D *Beauveria bassiana*: **A** conidia, 050408.22 (clade A); **B** conidia, 040511.24 (clade C); **C-D** conidiogenous cells, 040511.24. **E-F** *Beauveria brongniartii:* **E** conidia, 060510.1; **F** conidiogenous cells, 060510.1. Scale bars indicate 5 μm in A, B, C, E; and 10 μm in D, F.

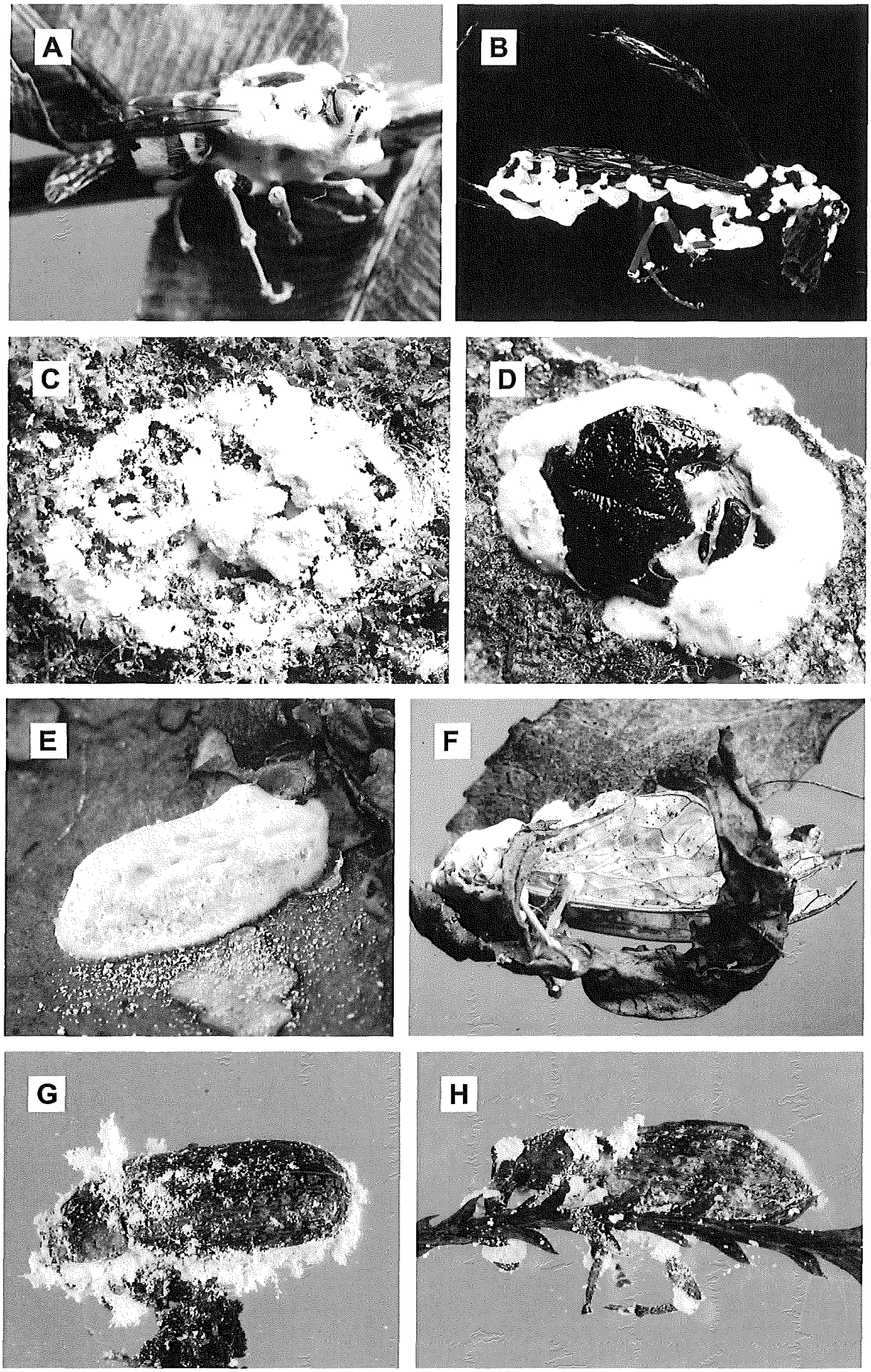


Figure 3.3. A-H *Beauveria malawiensis:* **A** 030509.4, **B** 000408.1 on Hymenoptera; **C** 030506.1, on Coleoptera; **D** 030509.15, **E** 060415.27, **F** 030507.1, on Hemiptera; **G** 060508.3, **H** 060511.3, on Coleoptera.

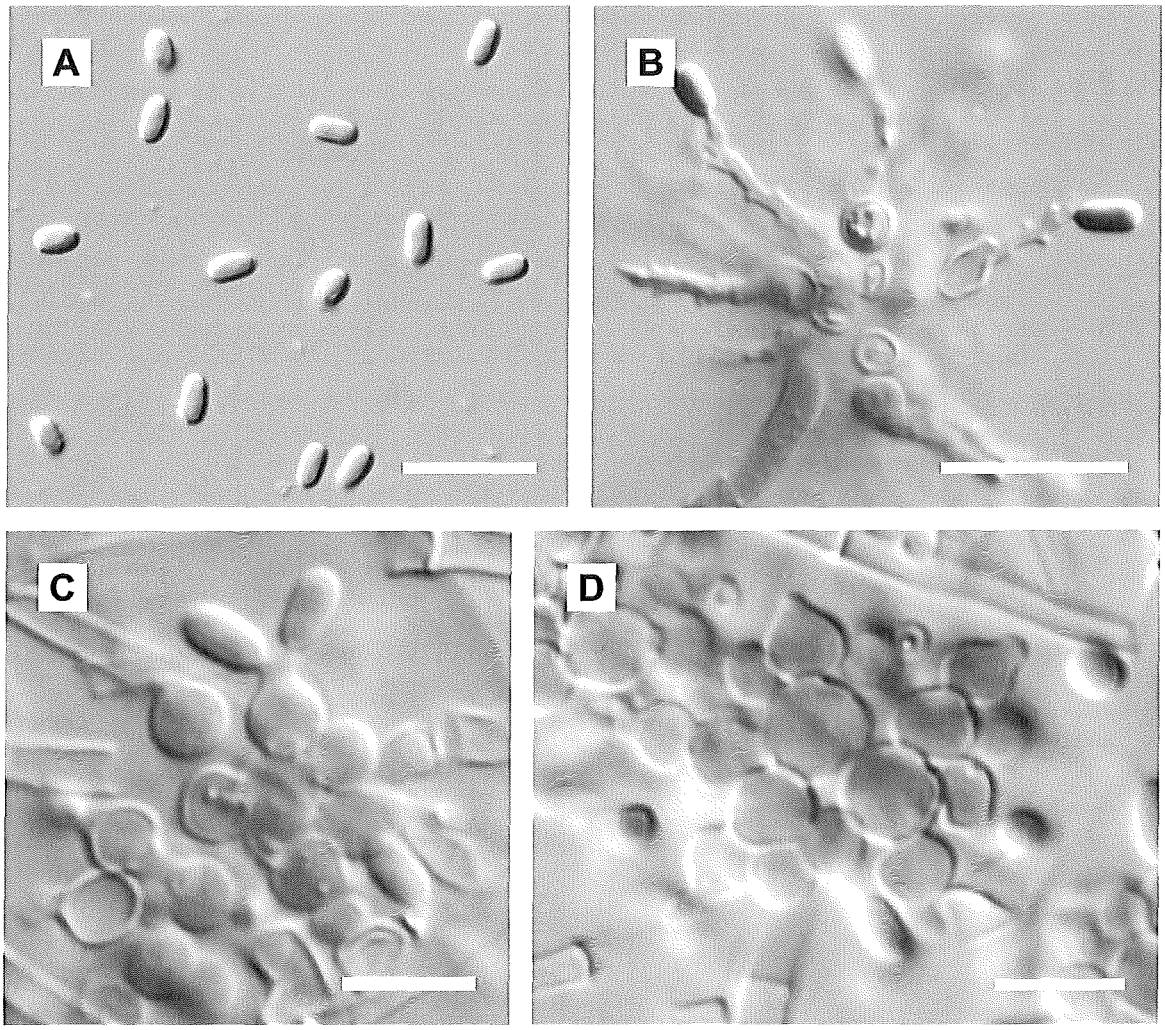


Figure 3.4. A-D *Beauveria malawiensis*: **A** conidia, 060512.5; **B** conidiogenous cells, 060511.4; **C-D** conidiogenous cells, 060415.51. Scale bars indicate 10 μm in A, B; 5 μm in C, D.

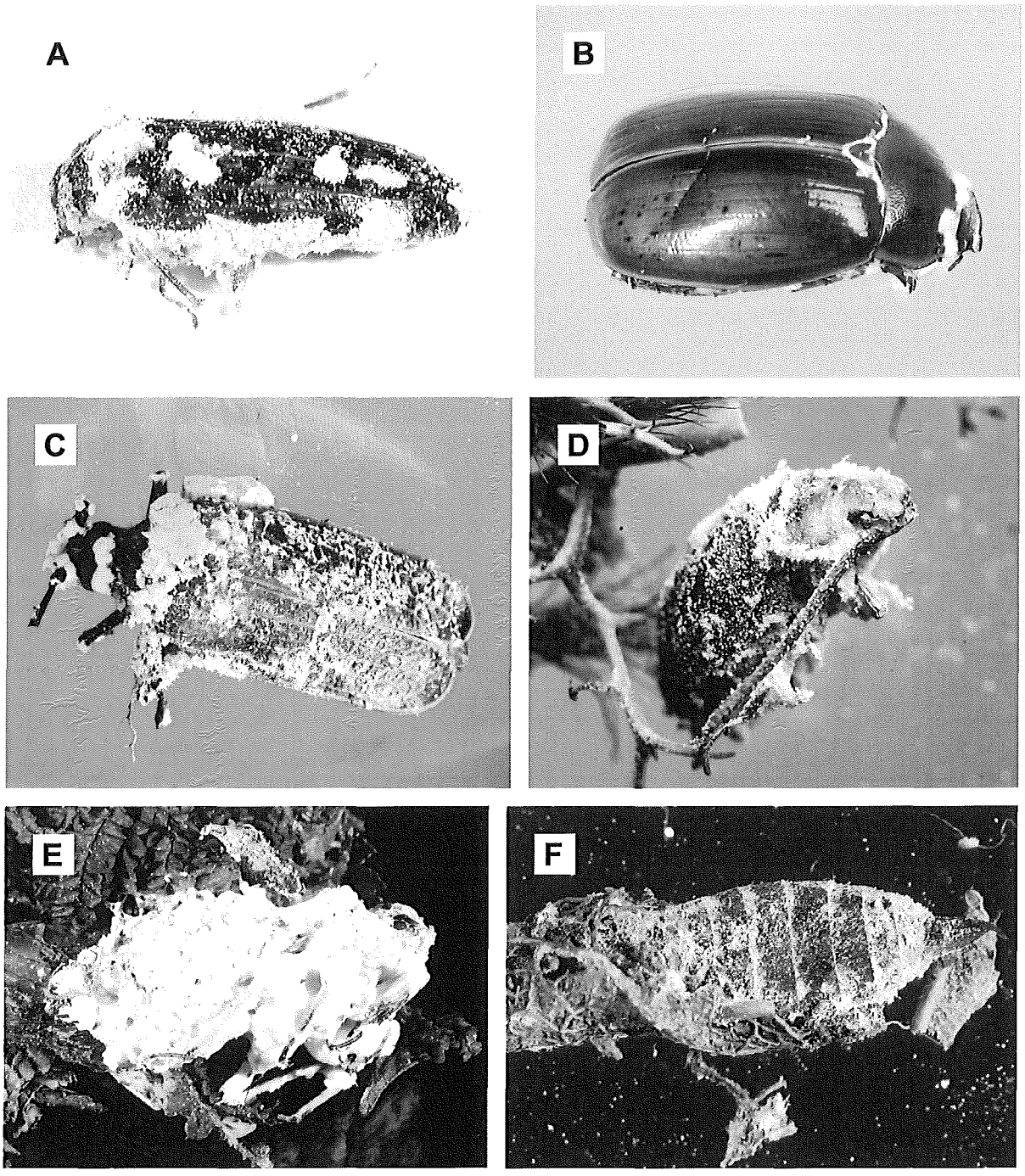


Figure 3.5. A-F *Beauveria caledonica*: **A** 030422.2, **B** 030509.18, **C** 060415.3, **D** 050406.4, on Coleoptera; **E** 050408.2, on Hemiptera; **F** 040513.17, on Dermaptera.

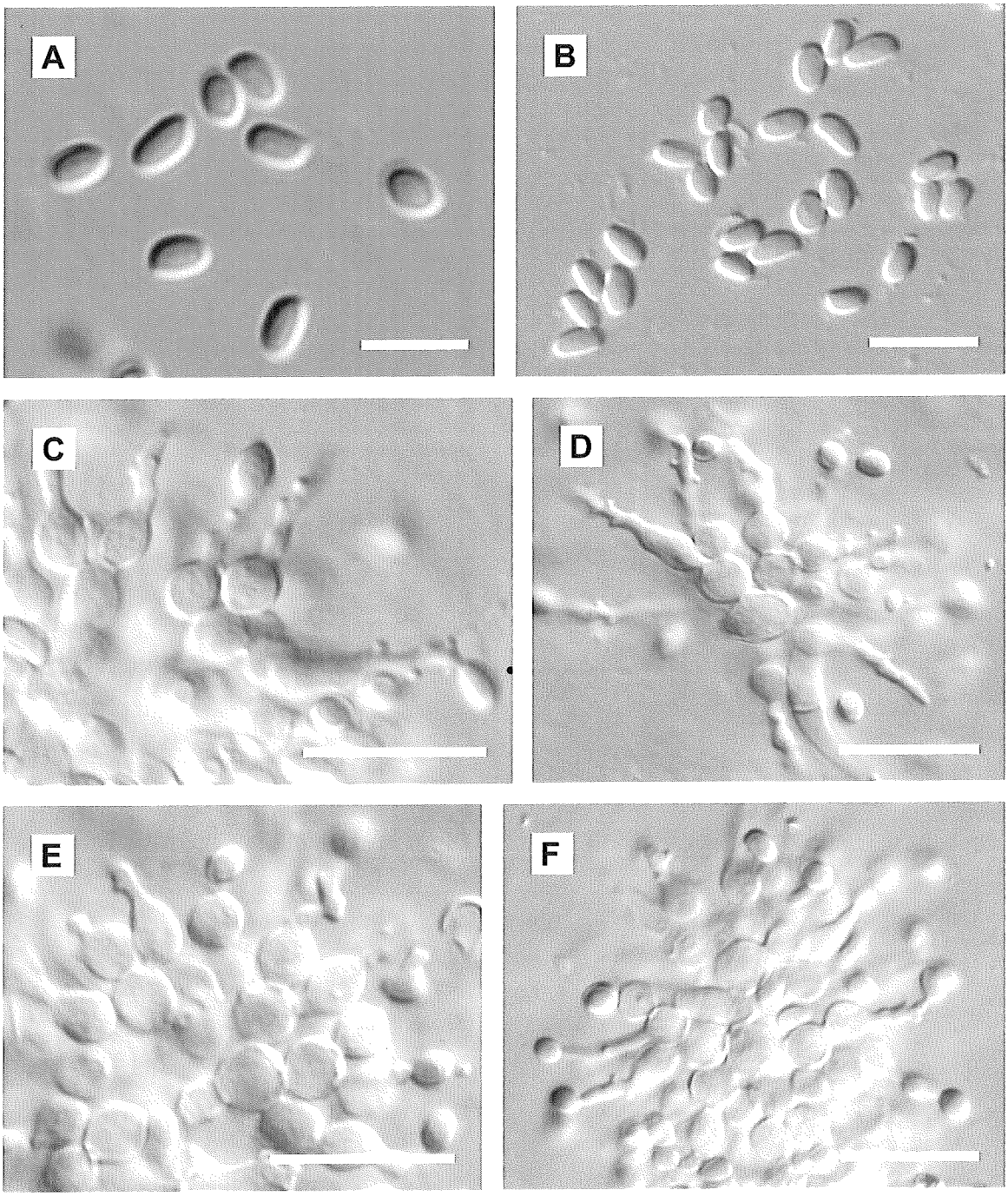


Figure 3.6. A-F *Beauveria caledonica*: **A** conidia, 30422.2, **B** conidia from 1/4 SDYA culture NC94,**C-F** conidiogenous cells,060415.3.

3.3.3 Phylogenetic analyses

Sequences from the ITS1-5.8S-ITS2 region using primers AB28 and TW81 were approximately 534 nucleotides in length. Sequences were trimmed at each end to match the shortest Genbank sequences included in the analyses. The final ITS dataset consisted of 506 aligned positions, of which 52 were parsimony-informative sites. The optimal tree from the neighbour-joining analysis of the full ITS dataset is shown in Fig. 3.7. Maximum parsimony (MP) analysis of the smaller ITS dataset yielded 902 equally parsimonious trees with a length of 89 steps. Bayesian likelihood analysis was conducted using the GTR+I+G model (general time reversible model with a gamma distribution and a proportion of invariable sites). The consensus tree from Bayesian analysis showed no significant conflict with the trees from MP analysis. One of the most parsimonious trees from the maximum parsimony analysis of the ITS region is shown in Fig. 3.8 with MP bootstrap values (BS) and posterior probabilities (PP) from the Bayesian analysis.

Partial EF1-α sequences obtained using the primers 1777F and 2218R consisted of approximately 529 nucleotides. These were trimmed in the final alignment to match the shortest included Genbank sequence. The final EF1-α dataset had 470 aligned positions with 30 parsimony-informative sites. The optimal tree from the neighbour-joining analysis of the full EF1-α dataset is shown in Fig. 3.9. Maximum parsimony analysis of the smaller EF1-α dataset yielded 1012 equally parsimonious trees with a length of 61 steps. Bayesian likelihood analysis was conducted using the GTR+I+G model. The consensus tree from Bayesian analysis showed no significant conflict with the trees from MP analysis. One of the most parsimonious trees from the maximum parsimony analysis of EF1-α is shown in Fig. 3.10 with MP bootstrap values (BS) and posterior probabilities (PP) from the Bayesian analysis.

Neighbour-joining, maximum parsimony and Bayesian analyses of the EF1-α dataset generally supported the results from the ITS analyses, but usually with lower branch support for the terminal clades. However, the ITS and EF1-α phylogenies showed significant conflict in their grouping of several *B. caledonica* isolates and the datasets were not considered suitable for combined analysis.

In most cases analysis of the ITS and partial EF1-α regions tended to confirm species identifications based on conidial morphology. *Beauveria bassiana* isolates clustered in the two main lineages (clades A and C) identified by Rehner and Buckley (2005). Differing levels of support for clade C were provided by EF1-α (84% NJ BS, 75% MP BS, 53% PP) and ITS (98 % NJ BS, 69% MP BS, unsupported in Bayesian). Analyses of the EF1-α region showed limited

support (54% NJ BS, 32% MP BS, 53% PP) for clade A compared with ITS (95% NJ BS, 71% MP BS, 100% PP).

A single isolate identified from morphology as *B. bassiana* grouped with *B. brongniartii* in the NJ and MP analyses of ITS (99% NJ BS, 90% MP BS) and EF1-α (84% NJ BS, 64% MP BS). Bayesian analysis of each region failed to resolve *B. brongniartii*, with the three included sequences forming a polytomy at a basal node. The identification of *B. malawiensis* was strongly supported from analysis of both ITS (99% NJ BS, 99% MP BS, 100% PP) and EF1-α (94% NJ BS, 88% MP BS, 98% PP). In the ITS phylogeny New Zealand *B. caledonica* isolates formed two distinct well-supported clades. One group corresponded with Scottish (ARSEF 2567) and Swiss (ARSEF 1567) isolates of *B. caledonica* (97% NJ BS, 90% MP BS, 98% PP) while the other group was closer to a South American isolate ARSEF 2251 (89% NJ BS, 70% MP BS, 55% PP). In contrast, partial EF1-α sequences grouped all of the New Zealand *B. caledonica* with ARSEF 2251 (62 % NJ BS, 71% MP BS, 65% PP) while ARSEF 1567 and ARSEF 2567 formed a distinct and strongly supported (67% NJ BS, 82% MP BS, 95% PP) clade.

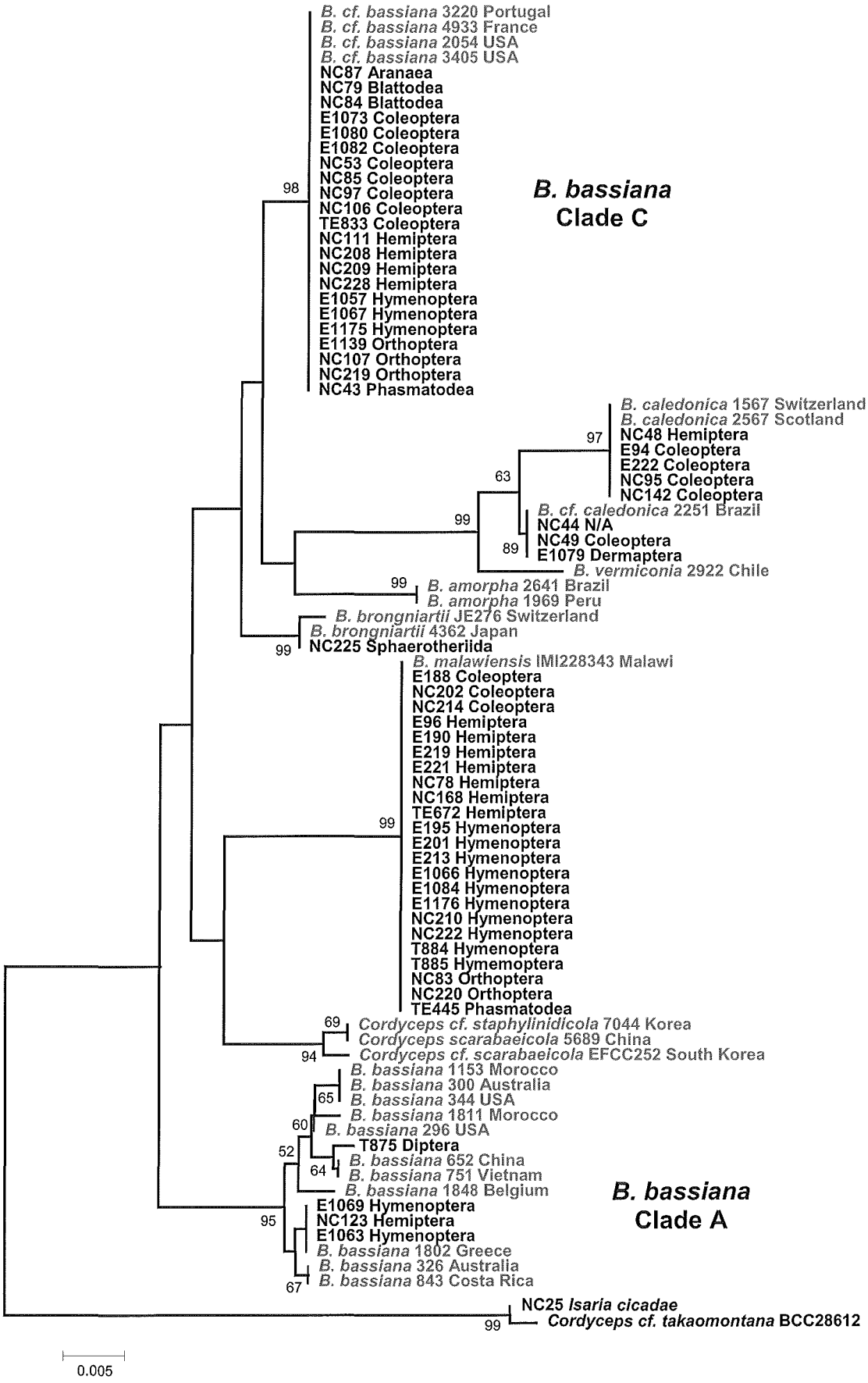


Figure 3.7 Optimal tree from neighbour-joining analysis of ITS sequences from *Beauveria* isolates. Genbank accession numbers for overseas isolates (in red) are given in Table 3.3. Bootstrap values (1000 replicates) and posterior probabilities ≥50% from Bayesian likelihood analysis are labelled at each branch, respectively.

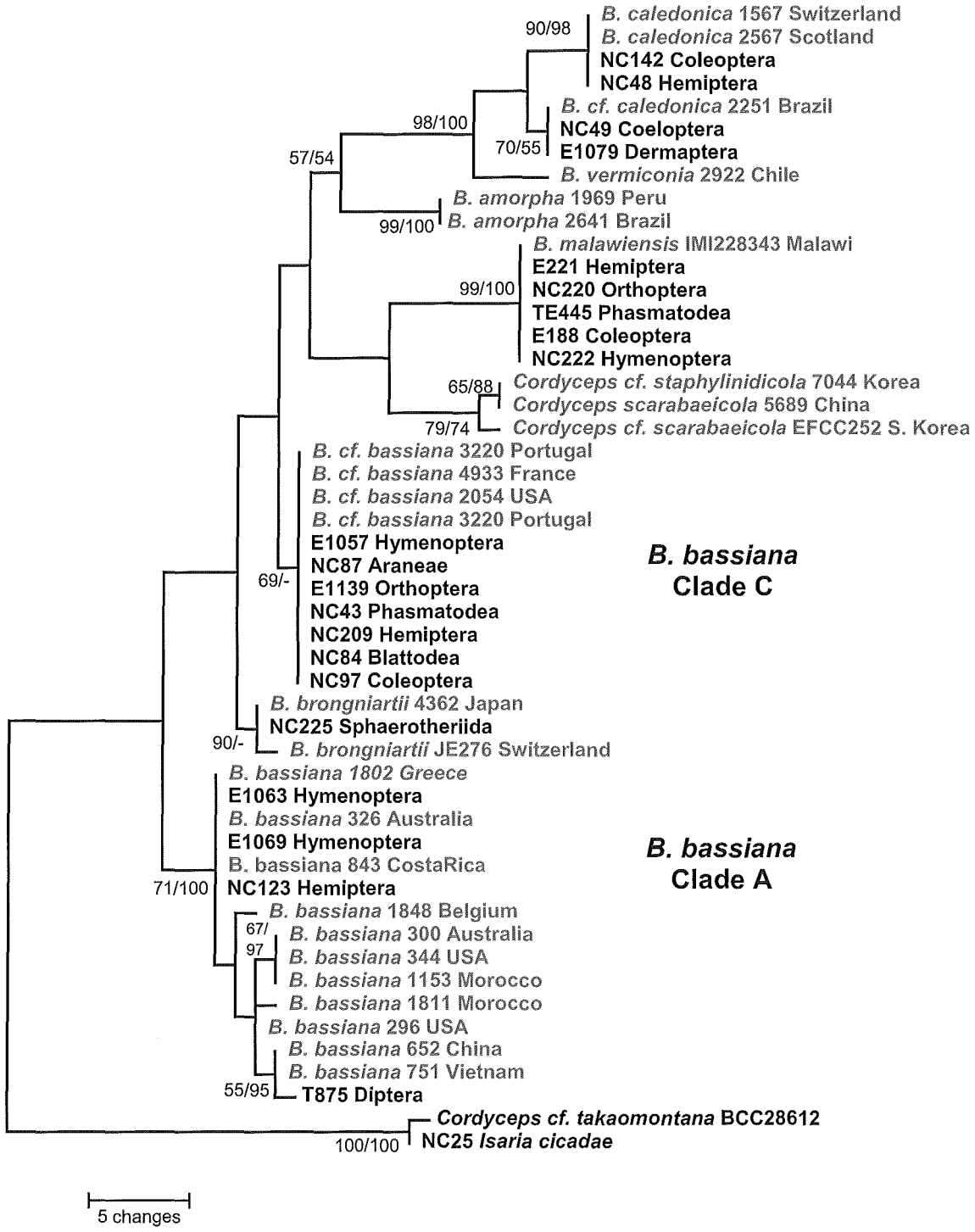


Figure 3.8 One of the shortest trees from maximum parsimony analysis of ITS sequences from *Beauveria* isolates. Genbank accession numbers for overseas isolates (in red) are given in Table 3.3 Bootstrap values (1000 replicates) and posterior probabilities ≥50% from Bayesian likelihood analysis are labelled at each branch, respectively.

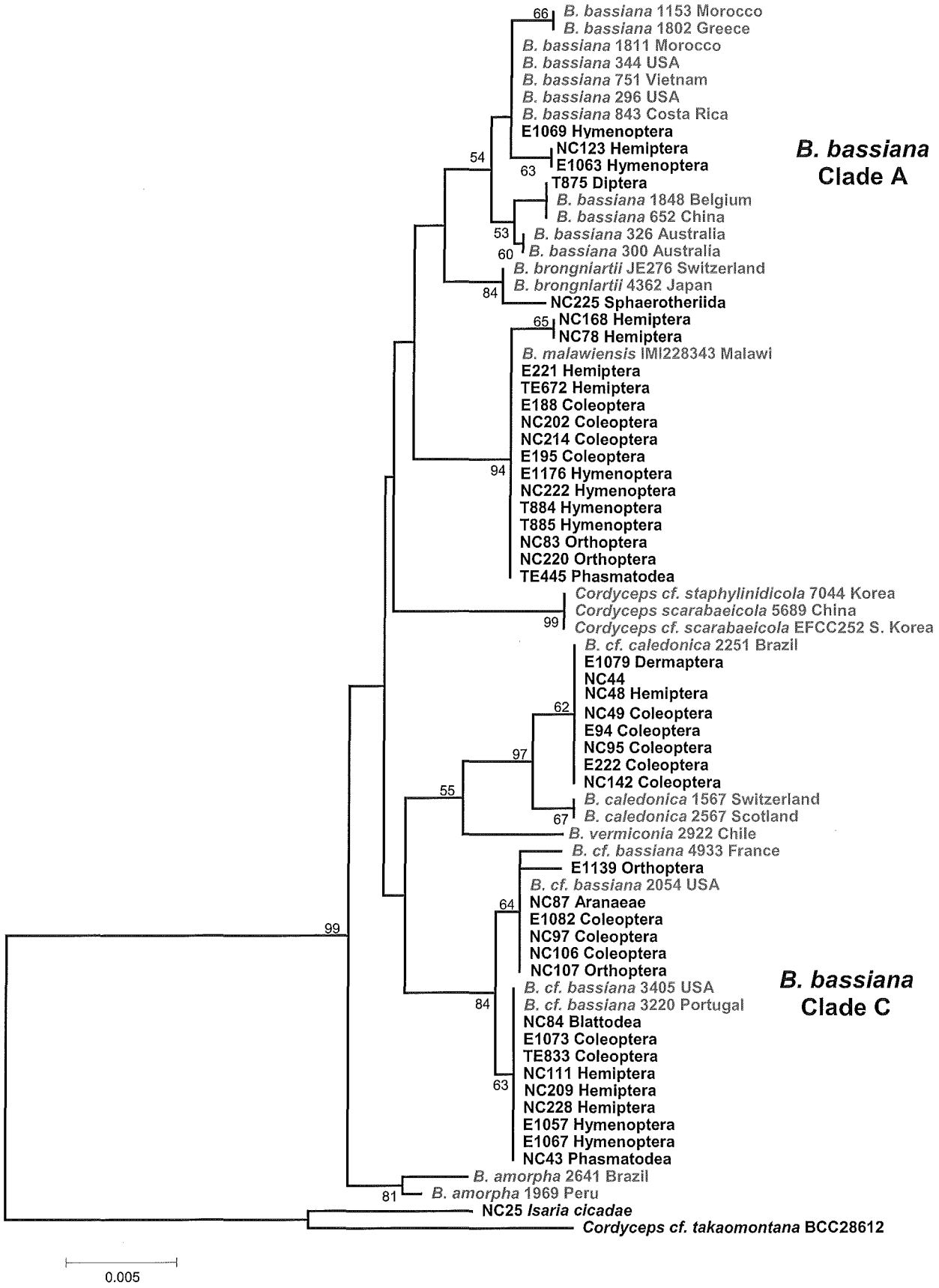


Figure 3.9 Optimal tree from neighbour-joining analysis of EF1-α sequences from *Beauveria* isolates. Genbank accession numbers for overseas isolates (in red) are given in Table 3.3. Bootstrap values (1000 replicates) and posterior probabilities ≥50% from Bayesian likelihood analysis are labelled at each branch, respectively.

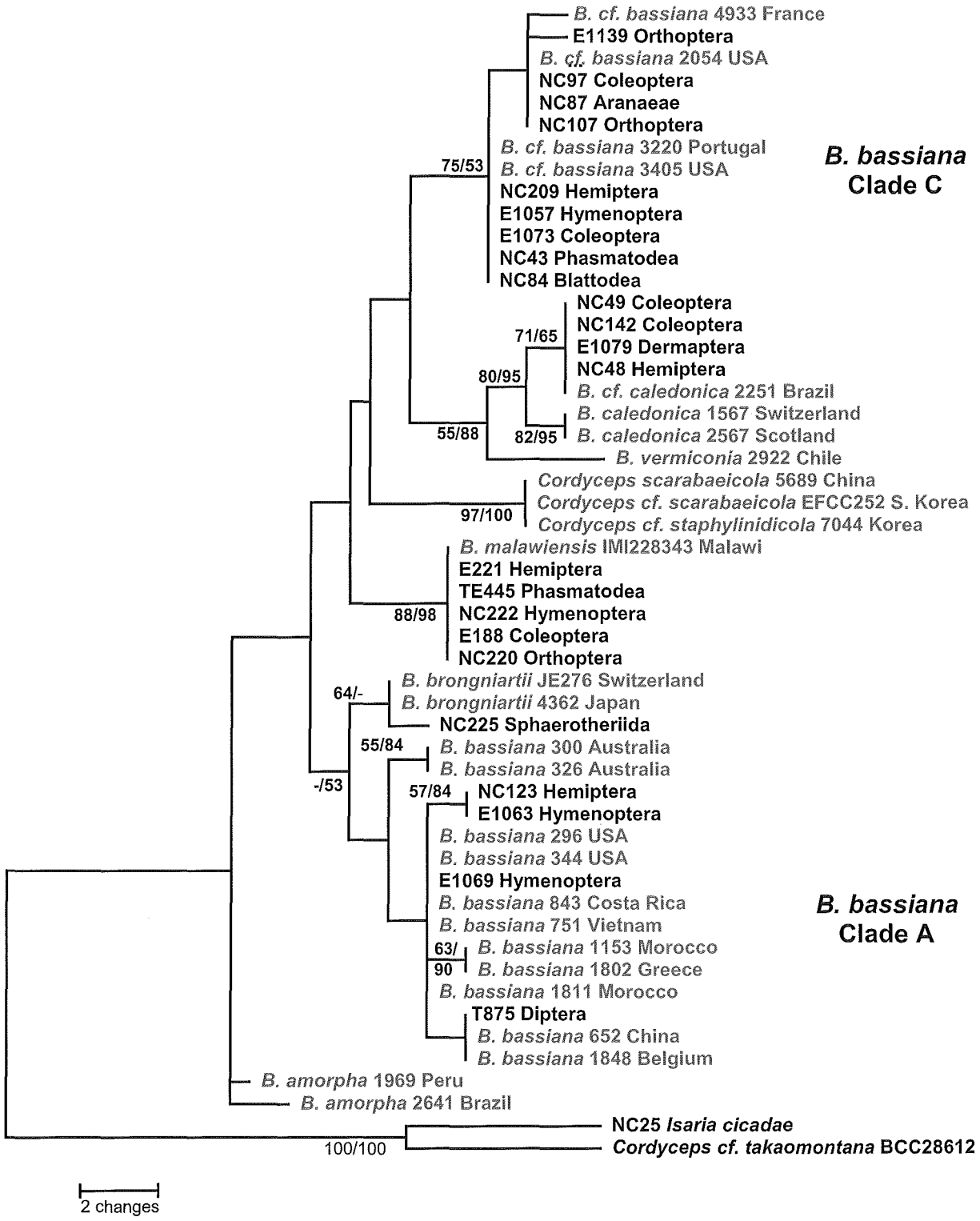


Figure 3.10 One of the shortest trees from maximum parsimony analysis of EF1-α sequences from *Beauveria* isolates. MP bootstrap values and posterior probabilities ≥50% from Bayesian likelihood analysis are labelled at each branch, respectively. Genbank accession numbers for overseas isolates (in red) are given in Table 3.3.

3.3.4 *Tenebrio molitor* bioassays

Bioassays confirmed that all tested strains of *B. bassiana* and *B. malawiensis* isolated from coleopteran, hemipteran, and hymenopteran hosts were pathogenic towards *T. molitor* larvae. All *Beauveria* strains caused significant mortality of *T. molitor* when compared to the controls after 12 days. Control mortality ranged between 0% and 5% after 12 days (Fig 3.11A-C). Mortality trends were similar across all of the tested fungi with most larvae dying between 5 and 12 days.

Coleopteran isolates

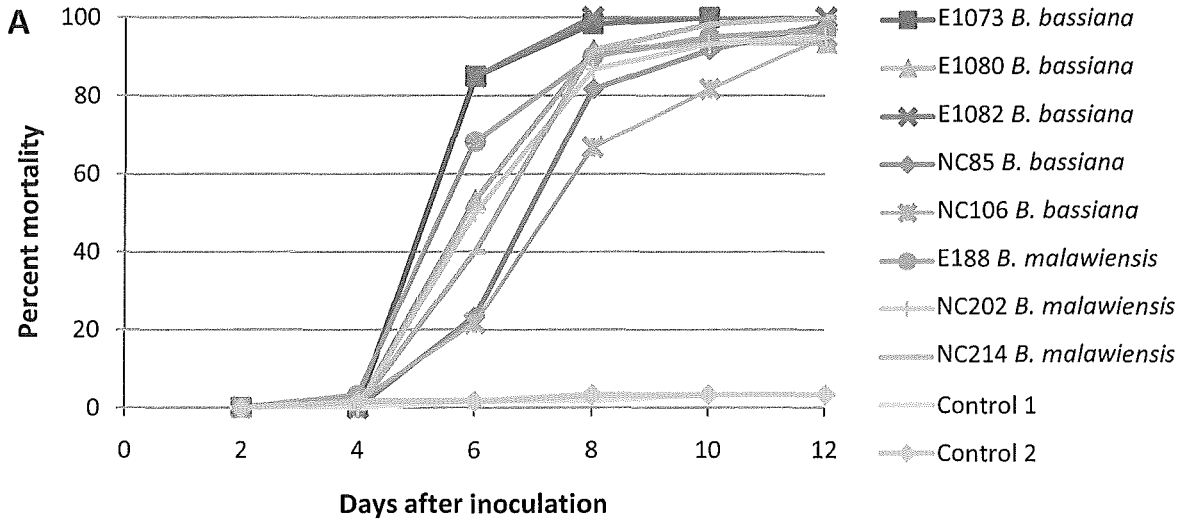
Mean mortality caused by isolates from Coleoptera ranged from 93% to 100% after 12 days (Fig. 3.11A). Mean LT_{50} values ranged from 5.14 to 7.49 days with a total mean LT_{50} of 6.12 days. Isolate NC106 (*B. bassiana*) had significantly higher LT_{50} values compared to all other isolates (Fig 3.12A). Total means for each species were 6.17 days for *B. bassiana* and 6.03 days for *B. malawiensis*. No significant difference was found between the total mean LT_{50} values from each species.

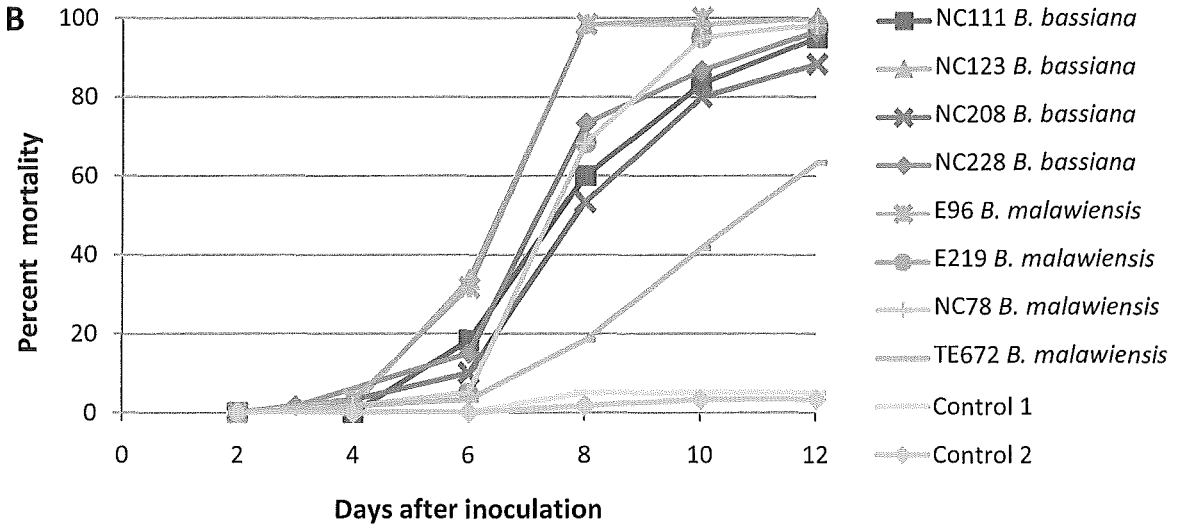
Hemipteran isolates

Mean mortality caused by isolates from Hemiptera ranged from 63% to 100% after 12 days (Fig. 3.11B). Mean LT_{50} values ranged from 6.43 to 10.8 days with a total mean of 7.54 days. Isolate TE672 (*B. malawiensis*) had significantly higher LT_{50} values compared to all other isolates (Fig 3.12B). Total means for each species were 7.09 days for *B. bassiana* and 7.98 days for *B. malawiensis*. Total mean LT_{50} values were significantly higher for *B. malawiensis*.

Hymenopteran isolates

Mean mortality caused by isolates from Hemiptera ranged from 67% to 100% after 12 days (Fig. 3.11C). Mean LT_{50} values ranged from 6.98 to 11.13 days with a total mean of 8.38 days. Isolates E1057 and E1069 (both *B. bassiana*) had significantly higher LT_{50} values compared to all other isolates (Fig. 3.12C). Total means for each species were 8.85 days for *B. bassiana* and 7.91 days for *B. malawiensis*. Total mean LT_{50} values were significantly higher for *B. bassiana*.





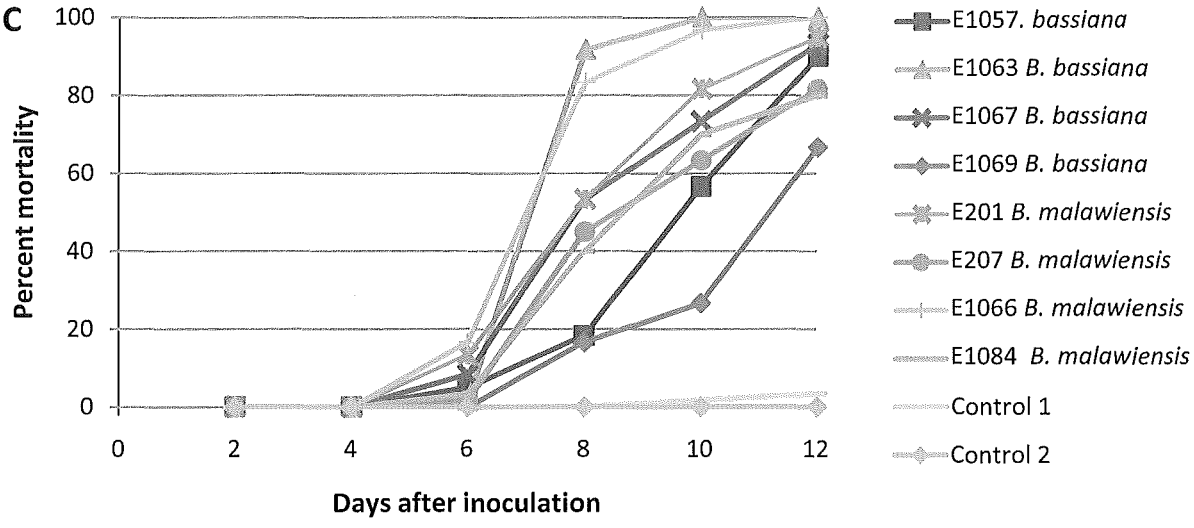


Figure 3.11 Cumulative mortality of *Tenebrio molitor* larvae after inoculation with *Beauveria* species isolated from A) Coleoptera; B) Hemiptera; C) Hymenoptera. Each graph shows means (n=60) from two replicate bioassays.

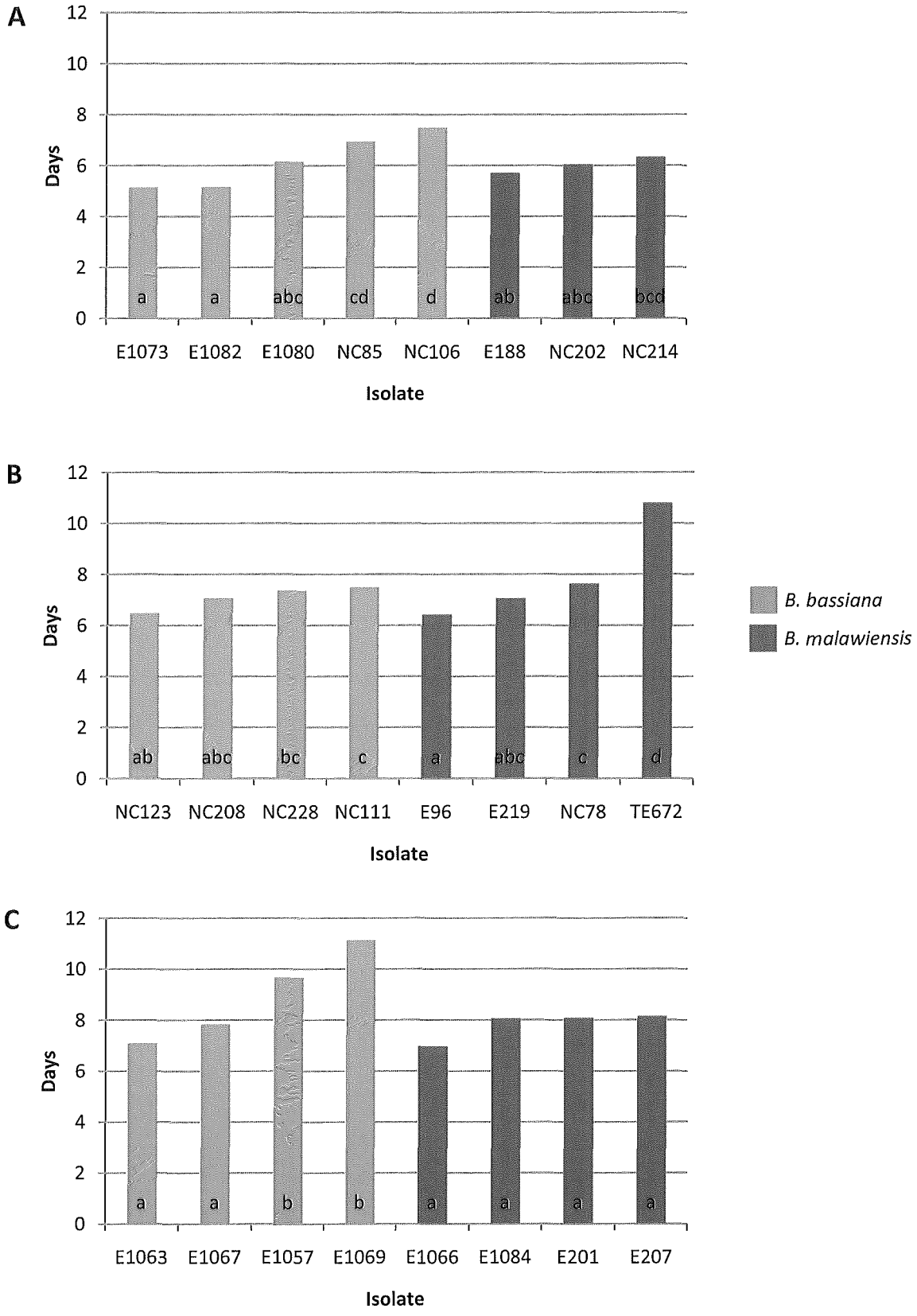


Figure 3.12 Mean LT_{50} values from bioassays of *Tenebrio molitor* larvae with *Beauveria* species isolated from: A) Coleoptera; B) Hemiptera; C) Hymenoptera. Each graph shows means (n=60) from two replicate bioassays. Means with the same letter in each graph are not significantly different at $P < 0.05$.

3.3.5 *Vespula vulgaris* bioassay

Bioassays confirmed that all tested strains of *B. bassiana* and *B. malawiensis* were pathogenic towards *V. vulgaris* larvae. All isolates caused significant mortality when compared to the controls after 10 days. Mortality ranged from 93 to 100% after 10 days (Fig. 3.13A). There was no mortality in control larvae. Mean LT_{50} values ranged from 4.7 days to 6.02 days with a total mean of 5.46 days. A significant difference was found between the most virulent (*B. bassiana* E1067) and least virulent (*B. malawiensis* E1066) isolates (Fig. 3.13B). Total means for each species were 5.13 days for *B. bassiana* and 5.79 days for *B. malawiensis*. Total mean LT_{50} values were significantly higher for *B. malawiensis*.

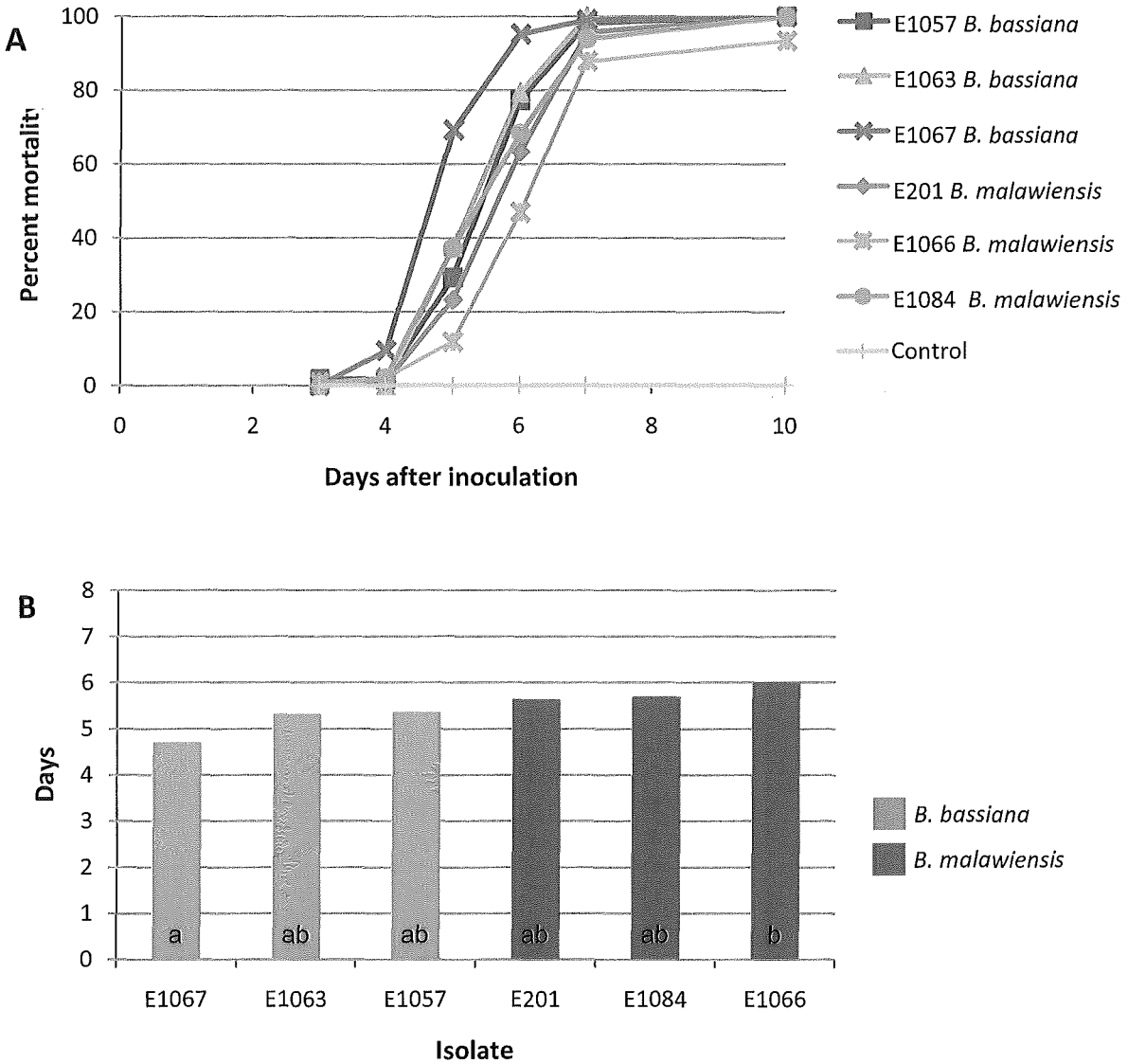


Figure 3.13 A) Cumulative mortality of *Vespula vulgaris* larvae after inoculation with *Beauveria* species; B) Mean LT_{50} values from *Vespula vulgaris* bioassay. Means with the same letter are not significantly different at $P < 0.05$.

3.4 Discussion

Beauveria species were common in New Zealand native forests and were collected from sites in all of the five main regions in this study (Tables 3.4, 3.6, 3.7). Insects were the most frequent hosts, with collections including representatives from Blattodea, Coleoptera, Dermaptera, Hemiptera, Hymenoptera, Orthoptera and Phasmatodea. Members of Hymenoptera (mainly *Vespula* spp.) were the most commonly collected hosts (33%), followed by species in Coleoptera (24%) and Hemiptera (16%, mainly Cicadidae). *Beauveria* species were also found on two non-insect arthropods in Aranaea and Diplopoda (Table 3.5). *Beauveria bassiana* and *B. malawiensis* appeared to be the main *Beauveria* species in native forest, with each sharing a broad host range. Fewer collections of *B. brongniartii* and *B. caledonica* were made, which may reflect more stringent host requirements of these species.

Beauveria bassiana, *B. malawiensis*, and *B. caledonica* could be readily distinguished by their conidial morphology (Tables 3.8-3.11), with sequence data from the ITS and EF1-α regions (Figs. 3.7-3.10) generally supporting identifications based on conidial form. However, conidial morphology did not fully indicate the phylogenetic affiliations of all isolates. Two previously reported phylogenetic species within *B. bassiana* were identified but could not be reliably separated by morphological data. Similarly, a *B. brongniartii* isolate (NC225) did not show the typical morphology of this species and could only be identified from molecular data. These results confirm the necessity of molecular-level characterisation for unambiguous identification of *Beauveria* species.

Beauveria bassiana was isolated from insect hosts in Blattodea, Coleoptera, Hemiptera, Hymenoptera, Orthoptera, and Phasmatodea. Several collections represent new host records for *B. bassiana* in New Zealand: *Stethaspsis suturalis* (Coleoptera), *Phaulacridium marginale* (Orthoptera), and *Celatoblatta* spp. (Blattodea). *B. bassiana* has not been previously reported in this country from any Blattodean or Orthopteran hosts. A single collection of a spider infected by *B. bassiana* represents an uncommon host association for this species. Most records of *B. bassiana* from arachnids are associated with mites (Chandler *et al.* 2000; Li 1998), and only one previous record of the species infecting spiders could be found (Petch 1931).

Specimens identified as *B. bassiana* typically had globose to subglobose conidia (Fig. 3.2A), measuring from 1.6-2.6 × 1.3-2.4 µm on the host and 1.8-3.0 × 1.6-2.8 µm in culture. Conidial sizes were comparable to those recorded for New Zealand (Glare *et al.* 1996a; Glare & Inwood 1998; Townsend *et al.* 1995) and overseas isolates (*e.g.* de Hoog 1972; Mugnai *et al.* 1989; Rehner & Buckley 2005). No significant correlation was found between conidial size and host.

Sequences from the ITS and EF1-α region separated *B. bassiana* isolates into the two phylogenetic species (clade A and clade C) identified by Rehner & Buckley (2005), confirming an earlier report that both taxa are present in New Zealand (Reay *et al.* 2007). Generally, representatives of each group were morphologically indistinguishable (*e.g.* Fig. 3.2A-B) and had overlapping ranges of conidial size. However, one clade A isolate (NC123) had slightly larger conidia than *B. bassiana* species identified as clade C in the phylogenetic analyses. Rehner & Buckley (2005) also reported larger conidia in this clade, although similarly the difference was not consistently shown in all isolates.

Beauveria bassiana isolates that grouped in clade C showed no variation in ITS sequences and were identical with the European and North American representatives included in the analyses. Slightly more variation was seen in the EF1-α region, but differences were limited to changes at only one or two nucleotide positions. Clade A isolates were more variable in both ITS and EF1-α sequences. Isolates E1063, E1069 and NC123 had similar ITS and EF1-α sequences, while T875 was more divergent and differed by a total of seven nucleotides over the two regions. These differences suggest that the two groups of isolates have different origins in New Zealand and may reflect the outcome of separate historical dispersal events (see Rehner & Buckley 2005; Rehner *et al.* 2006b). Sequences from isolate T875 consistently grouped with those from a Chinese isolate (ARSEF652) in the ITS and EF1-α phylogenies, suggesting a close relationship and shared evolutionary history of these isolates.

Currently, little information is available concerning the distribution of the two *B. bassiana* clades in New Zealand. The presence of both groups in native forests was first demonstrated by Reay *et al.* (2007) in association with *Platypus* spp. (Curculionidae: Coleoptera). In the present study, while representatives of both clades were also found in native forests, the majority of isolates grouped in clade C. In contrast, *B. bassiana* isolates from New Zealand pine plantations were all found to belong to clade A (Reay *et al.* 2008). These findings suggest that clade C may be restricted to undisturbed habitats in New Zealand. Populations of entomopathogenic fungi are thought to be particularly sensitive to disturbance effects from human land use (Barker & Barker 1998; Hywel-Jones 2001; Samson *et al.* 1988). Molecular characterisation of *B. bassiana* isolates from other modified environments (*e.g.* agricultural settings) in New Zealand may provide further information on any habitat preferences shown by each clade.

A *Beauveria* specimen (NC225) from the pill millipede *Procyliosoma tuberculatum* (Diplopoda) was initially identified as *B. bassiana* and formed globose-subglobose conidia (Fig. 3.2E) measuring 2.0-2.6 × 1.8-2.2 μm on the host and 2.1-3.0 × 1.9-2.7 μm in culture. However,

phylogenetic analysis identified the isolate as *B. brongniartii*, with ITS and EF1-α sequences each differing at a single nucleotide position from sequences of the closest overseas strain. While this species has generally been differentiated from *B. bassiana* by larger (>3μm long) ellipsoidal conidia (de Hoog 1972; Glare & Inwood 1998), in some cases strains that formed ellipsoidal conidia on the hosts were shown to produce only spherical conidia in culture (Mugnai *et al.* 1989; Townsend *et al.* 1995). In other studies, strains forming ellipsoidal conidia in culture were identified as *B. bassiana* based on DNA sequence data (Aquino de Muro *et al.* 2005; Rehner & Buckley 2005). Isolate NC225 provides another example of the difficulties associated with applying morphological criteria to distinguish between these two species. No other collections of *B. brongniartii* were made in this study and the species appears to be rare in native forests, despite earlier records of the fungus infecting a wide range of hosts in these habitats (Anon 2001-2009; Glare *et al.* 1993b).

Although millipedes (Diplopoda) are commonly infected by biotrophic fungal parasites in Laboulbeniales (Rossi & Weir 1998; Weir & Beakes 1995), there are few records of true fungal pathogens from these hosts. *Beauveria brongniartii* has not been previously recorded as infecting diplopods, although Petch (1931) collected *B. bassiana* from a millipede in Ceylon. *Verticillium griseum* (Gams 1971) and an undescribed *Lecanicillium* species (Kurihaya *et al.* 2008) have also been reported from millipedes. Significantly, two surveys aimed at finding potential biocontrol agents for millipede pests failed to find any entomopathogenic fungi among their natural enemies (Baker 1985; Brito 1994). Diplopods are known to secrete defence compounds with antifungal properties (Sierwald & Bond 2007), which could explain the lack of pathogenic fungi found in association with these invertebrates.

Beauveria malawiensis is recorded infecting insects in New Zealand for the first time. The species was easily distinguished from other *Beauveria* species by the characteristic straight, cylindrical conidia (Fig. 3.4A-C). Conidia from cultures of New Zealand isolates were of slightly different dimensions (3.0-4.8 × 1.1-2.0 μm) to those recorded in the original description of *B. malawiensis* (3.7-4.5 × 1.3-1.9 μm), but shared the typical globose conidiophores (Fig. 3.4C-D) and pink conidia in culture described for this species (Rehner *et al.* 2006a). No significant correlation was found between conidial size and host. ITS sequences were identical to those from the type specimen, as were most partial EF1-α sequences, apart from two isolates from Hemiptera which differed at a single nucleotide position. A previous report identified an isolate from a New Zealand pine forest soil as *B. malawiensis* based on analysis of a partial EF1-α sequence (Reay *et al.* 2008). Another New Zealand soil isolate identified variously as *B.*

brongniartii (Glare 2004) and *Cordyceps scarabaeicola* (Reay *et al.* 2007), also represents *B. malawiensis* based on the ITS sequence available for this isolate (Genbank DQ385618).

Rehner *et al.* (2006a) described *B. malawiensis* from a single culture isolated from the coleopteran species *Phoracantha semipunctata* (Cerambycidae) and there have been no subsequent records of this species from other hosts. The present study significantly extends the known host range of *B. malawiensis*. In native forests, the species was collected on hosts in at least ten different families in Coleoptera, Hemiptera, Hymenoptera, Orthoptera and Phasmatodea. Hymenopteran. *Vespula* species were particularly common as hosts of *B. malawiensis,* and large numbers of wasps killed by this species were observed at sites in Nelson and the Bay of Plenty.

Beauveria malawiensis was first described from an isolate which had been originally identified as *B. brongniartii* (Rehner *et al.* 2006a*).* The apparent rarity of *B. brongniartii* in native forests observed in this study suggests that many earlier records of this species in these habitats may have actually been *B. malawiensis*. This conclusion is also supported by the wide host range previously recorded for *B. brongniartii* in this country (Anon 2001-2009; Glare *et al.* 1993b), which tends to correlate with that of *B. malawiensis*. Examination of PDD herbarium specimens originally identified as *B. brongniartii* confirmed that these are all *B. malawiensis* or *B. bassiana*. Based on conidial morphology, the only PDD specimen likely to represent *B. brongniarti* is PDD25211 (received as *B. tenella*). However, the material examined consisted of a dried agar culture, and any identification must remain tentative considering previous reports of the variability of *B. bassiana* and *B. brongniartii* in culture (Aquino de Muro *et al.* 2005; Mugnai *et al.* 1989; Rehner & Buckley 2005; Townsend *et al.* 1995). While *B. brongniartii* seems to be rare in native forests, several isolates from scarabaeid hosts in New Zealand pastures appear to be 'authentic' *B. brongniartii* based on morphological and molecular data (Glare & Inwood 1998), so the species may be more common in agricultural habitats.

To investigate host specificity in *B. malawiensis* and *B. bassiana,* laboratory bioassays were conducted to examine the pathogenicity of strains isolated from various hosts towards the coleopteran species *Tenebrio molitor*. *B. bassiana and B. malawiensis* isolates from Coleoptera, Hymenoptera and Hemiptera were all shown to be pathogenic towards *T. molitor* larvae (Fig. 3.11). All of the isolates tested caused significantly higher mortality than the controls after 12 days. These results would seem to indicate that the examined strains of both *B. malawiensis* and *B. bassiana* are generalists with no strict host preference. However, differences in virulence as expressed by LT_{50} times were observed among isolates from each host group. Although isolates

from non-coleopteran hosts generally had similar virulence to those derived from Coleoptera, single isolates of *B. bassiana* from the hemipteran group and *B. malawiensis* from the hymenopteran groups each caused significantly lower mortality (Fig. 3.11) and had higher LT_{50} times (Fig. 3.12). Although this could suggest a degree of specialisation towards the hosts from which they were originally isolated, they could instead represent strains that are simply less virulent towards insects in general. Further studies testing comparative virulence towards a wide range of insect species are needed to more fully investigate any specific host preferences shown by individual isolates of these species.

The potential of *B. bassiana* and *B. malawiensis* strains for control of *Vespula* wasps was also examined. The latter species has not been previously tested against these insects. *B. bassiana* and *B. malawiensis* strains isolated from wasp hosts all caused significant mortality compared with the controls when tested against *V. vulgaris* larvae (Fig. 3.13A). Isolates varied in virulence towards *V. vulgaris*. Shorter LT_{50} times were shown by *B. bassiana* isolates indicating that these strains may make the best candidates for wasp control (Fig. 3.13B). Further characterisation of the efficacy of these strains against *Vespula* species is suggested.

Beauveria caledonica was also isolated from insects in native forests. The species has only been previously recorded in New Zealand in association with Coleoptera in pine plantations (Glare *et al.* 2008; Reay *et al.* 2008). While most collections of *B. caledonica* from native forest were also from Coleoptera, single collections of an infected cicada and a dermapteran (earwig) species were also made. The species has not been previously recorded from non-coleopteran hosts.

Morphologically, *B. caledonica* specimens were characterised by ellipsoidal to cylindrical conidia, measuring 2.2-3.3 × 1.1-1.6 µm on the host (Fig. 3.6A) and 2.8-5.1 ×1.0-2.1 µm in culture (Fig. 3.6B), closely matching the description by Bisset & Widden (1988). While conidial dimensions tend to overlap those of *B. malawiensis*, conidia of *B. caledonica* were often distinctively flattened on one side or slightly curved, sometimes with an almost reniform appearance. Conidia of *B. amorpha* also have a similar shape but are larger, measuring 3.5-5 x 1.5-2.0 µm on the host and 5-6 x 1.5-1.7 µm in culture (Samson & Evans 1982). In the ITS phylogeny, New Zealand isolates of *B. caledonica* formed two separate lineages. One group had identical ITS sequences Scottish (ARSEF 2567) and Swiss (ARSEF 1567) strains of *B. caledonica* while those of the other group were identical to a South American strain, ARSEF 2251. In contrast, EF1-α sequences grouped all of the New Zealand isolates with ARSEF 2251. No consistent morphological differences were observed between the two groups. Glare *et al.* (2008) also reported New Zealand *B. caledonica* isolates with ITS identical to ARSEF 2567 and

ARSEF 1567, and EF1-α identical to ARSEF2251. Rehner & Buckley (2005) suggested that ARSEF 2251 may represent a separate species from *B. caledonica* based on its differing ITS and EF1-α sequences and a slight difference in conidial size. However, until the taxonomic status of this strain is clarified it seems advisable to classify all of the New Zealand strains discussed here as *B. caledonica.*

Glare *et al.* (2008) suggested that *B. caledonica* may have been introduced to New Zealand from Britain with *Hylastes ater* and *Hylurgus ligniperda*, based on the occurrence of the fungus on similar hosts in both countries. In this case, the present study shows that *B. caledonica* has now become established in native forest, possibly through migration of infected hosts from pine forests. The discovery of two distinct ITS haplotypes in New Zealand strains suggests that two separate introductions of *B. caledonica* have taken place.

Increasing molecular evidence has shown that many elements of the New Zealand biota (see McDowall 2007; Perrie & Brownsey 2007), including fungal species (Moncalvo & Buchanan 2008; Moyersoen *et al.* 2003) have originated in New Zealand through long distance dispersal. This is particularly relevant to explaining geographically disjunct distributions of fungal taxa, as fungal spores have been shown to travel for thousands of kilometres on wind currents (Brown & Hovmoller 2002; McKenzie 2000). Entomopathogenic fungi may also migrate via living, infected insects; or through accidental transportation of diseased insects by human activities (Bidochka & Small 2005). Future studies using additional, higher resolution molecular markers (*e.g.* Rehner *et al.* 2006b) may provide insight into the phylogeographic history and likely origins of *Beauveria* species in New Zealand.

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CHAPTER FOUR: THE GENUS *ISARIA* IN NATIVE FORESTS

4.1 Introduction

The genus *Isaria* has had a complex taxonomic history. The name *Isaria* was first used by Hill in 1791 for three species that are now recognised as representing a myxomycete, a basidiomycete and a rust (Petch 1934). Throughout the nineteenth and early twentieth century, many species were added to the genus, which ultimately came to include over 200 species of mainly entomopathogenic and mycoparasitic fungi (Hodge *et al.* 2005). Generally, species were classified in *Isaria* based on the presence of simple or branched synnemata producing one-celled hyaline conidia, with no consideration given to differences in conidiogenous structures (Mains 1955). Petch (1934) also noted that in some cases species were also included in the genus solely on the basis of their association with insects.

Members of *Isaria* were later redistributed amongst diverse fungal groups, with entomopathogenic species transferred to several genera including *Akanthomyces*, *Gibellula*, *Hirsutella*, *Hymenostilbe* and *Spicaria* (*e.g.* Speare 1920; Petch 1932a, 1932b, 1936, 1937; Mains 1950). Clements & Shear (1931) proposed the entomopathogenic *Isaria farinosa* as the type species for *Isaria*, and this decision was also supported by Mains (1955). However, Petch (1934) reviewed the early nomenclatural history and identified varying concepts of the genus among early authors, leading to confusion over the correct typification. Petch suggested that *Isaria* should not be used as a generic name and followed Vuillemin who had earlier regarded *I. farinosa as* a member of *Spicaria* (Brown & Smith 1957).

The genus *Spicaria* was formerly used to accommodate species with verticillate conidiophores and phialides, bearing chains of conidia. However, as noted by Vuillemin (1912) and Owen (1919), these characteristics differed from the original description of the genus which had been misinterpreted by subsequent authors. Hughes (1951) suggested that because of this taxonomic confusion *Spicaria* should be abandoned and transferred two species to the similar but little known genus *Paecilomyces,* established by Bainier in 1907 for the thermophilic species *P. variotii*. *Paecilomyces* was described as resembling *Penicillium* but is primarily distinguished by short cylindrical phialides tapering into long thin necks (Pitt & Hocking 1985). The genus was monographed by Brown & Smith (1957), who followed Hughes (1951) and included many species previously classified in *Spicaria* and *Isaria.* Onions & Barron (1967) also included species with solitary phialides in *Paecilomyces*, but most of these were later transferred to *Acremonium* (Gams 1971).

Samson (1974) restricted *Paecilomyces* to species with verticillate conidiophores producing divergent whorls of branches and phialides. Phialides were characterised by a cylindrical or inflated base and a long distinct neck, bearing chains of hyaline, one-celled conidia. Several species classified in *Paecilomyces* by Brown & Smith (1957) with deviating conidiogenous structures (*e.g.* awl-shaped phialides or basitonous branching) were assigned to other genera. Samson also separated *Paecilomyces* into two sections: sect. *Paecilomyces* and sect. *Isarioidea.* Section *Paecilomyces* included *P. variotii* and other thermophilic or thermotolerant fungi, many of which are commonly found as food spoilage organisms. *Paecilomyces farinosus* and other invertebrate-pathogenic species were placed in section *Isarioidea* which included all species with flask shaped phialides and catenate conidia formerly classified in *Isaria.* Several new species from insects collected in tropical forests in Ghana (see Evans 1974) were also included in this section. In addition to being ecologically distinct, the two sections within *Paecilomyces* recognised by Samson (1974) were also characterised by disparate teleomorphic affiliations, indicating that the genus was polyphyletic and did not reflect a natural taxonomic group. Species in section *Paecilomyces* were known to produce teleomorphs in the eurotialean genera *Byssochlamys, Talaromyces* and *Thermoascus* (Brown & Smith 1957; Samson 1974; Stolk & Samson 1972) , while entomopathogenic species in section *Isarioidea* had been linked with the hypocrealean genera *Cordyceps* and *Torrubiella* (*e.g.* Kobayasi 1941; Mains 1949; Petch 1937*).*

The phylogenetic placement of *Paecilomyces* section *Isarioidea* within the Hypocreales was confirmed for one member of the section, *P. tenuipes*, from analysis of 18S (Fukatsu *et al.*1997) and 28S rDNA sequences (Nikoh & Fukatsu 2000; Suh *et al.* 1998,). Oborník *et al.* (2001) analysed 28S sequences from four invertebrate-pathogenic species in sect. *Isarioidea* and demonstrated that this section was polyphyletic. Phylogenetic relationships based on 18S sequences among a larger group of *Paecilomyces* species were examined by Luangsa-ard *et al.* (2004) who included representatives from both sections. The genus was found to be polyphyletic at the order level; species in section *Paecilomyces* were shown to form a group within the Eurotiales, while those in section *Isarioidea* grouped in Hypocreales. The authors concluded that the genus *Paecilomyces* should be used only for eurotialean taxa related to the type species *P. variotii* and with a *Byssochlamys* teleomorph. The 18S phylogeny also confirmed the polyphyletic status of section *Isarioidea.* Luangsa-ard *et al.* (2005) further examined relationships in this group using ITS and β-tubulin tubulin sequences. Species in section *Isarioidea* were separated into four monophyletic groups. Significantly, a distinct clade of entomopathogenic species based around *P. farinosus* was recognized. These species were reclassified in *Isaria* (see Table 4.1) following a thorough review of the nomenclatural history

and proposal to conserve the genus as typified by *I. farinosa* (Hodge *et al.* 2005; Gams *et al.* 2005). Luangsa-ard *et al.* (2005) suggested that two additional species placed in section *Isarioidea* by Samson (1974), (*P. ramosus* and *P. xylariiformis*) probably also belong in *Isaria*, although cultures were not available for molecular analyses. It seems likely that other entomopathogenic *Paecilomyces* species with comparable morphology such as *P. breviramosus* (Bisset 1979b) and *P. rariramus* (Liang *et al.* 2003) should also be included in the genus.

Although a close phylogenetic affiliation with *Cordyceps* has been well established by molecular evidence (*e.g.* Luangsa-ard *et al.* 2004, 2005; Nikoh & Fukatsu 2000, 2001; Stensrud *et al.* 2005; Sung *et al.* 2007a), few specific teleomorph connections are known for the species currently assigned to *Isaria*. A link with *Cordyceps* was originally suggested in the mid-nineteenth century by Tulasne who stated that *I. farinosa* was the teleomorph of *Cordyceps militaris* (Gray 1858). This connection was later refuted by Petch (1936) who determined from ascospore isolations that *C. militaris* produces a '*Cephalosporium*' anamorph (now included in *Lecanicillium*; Zare & Gams 2001). *Isaria farinosa* has also been associated with the spider-pathogenic species *Torrubiella gonylepticida* (Petch 1937) and *T. pulvinata* (Mains 1949). Both species were described with *Spicaria* anamorphs that were synonymised with *P. farinosus* by Samson (1974). More recently, Pacioni & Frizzi (1978) demonstrated from cultural studies that *Isaria farinosa* is the conidial state of the rarely collected species *Cordyceps memorabilis*. However, the direct association of *I. farinosa* with a teleomorphic state has not been confirmed by any subsequent authors. The most convincingly demonstrated anamorph-teleomorph connection in *Isaria* appears to be that between *I. tenuipes* and *Cordyceps takaomontana*. The association was first made by Kobayasi (1941) who observed that *C. takaomontana* produced perithecial stromata concurrently with an *I. tenuipes* conidial state when grown on a rice medium and also occasionally on the host. A close relationship between the two taxa was also suggested from analysis of 18S rDNA sequences (Nikoh & Fukatsu 2001). The relationship was further substantiated by Luangsa-ard *et al.* (2005) who found that a Thai isolate provisionally identified as *C. takaomontana* grouped with *I. tenuipes* in both β-tubulin and ITS-based phylogenies.

Isaria species show varying degrees of host specificity (Table 4.1). The broadest host range is seen in *I. farinosa* and *I. fumosorosea*. Both species are most commonly associated with Lepidoptera but each also infects hosts in several insect orders (see Zimmermann 2008). Both species are known to infect mites (Acari) (Chandler *et al.* 2000), with *I. farinosa* also occurring on other arachnids including spiders (Aranaea), harvestmen (Opiliones) and pseudoscorpions (Chelonethida) (Cokendolpher 1993; Leatherdale 1970; Samson 1974; Samson & Evans 1977;

Sosnowska *et al.* 2004). Other members of the genus appear to have more restricted host ranges and in some cases are only found in association with a single host order or family. For example, *I. ghanensis* and *I. coleopterorum* have only been recorded as pathogens of Lepidoptera and Coleoptera, respectively, while *I. cicadae* is restricted to immature stages of the single family Cicadidae in Hemiptera.

Table 4.1. Host ranges of species accepted in *Isaria* by Luangsa-ard *et al.* (2005).

Species	Host range	References
I. amoenerosea	Chelonethida, Coleoptera, Lepidoptera, Hemiptera, Hymenoptera	Samson 1974; Samson & Evans 1977
I. cateniannulata	Coleoptera, Diptera, Lepidoptera, Hymenoptera	Liang 1981; Shimazu 2001
I. cateniobliqua	Lepidoptera	Liang 1981
I. cicadae	Hemiptera (Cicadidae)	Samson 1974; Liang *et al.* 2005
I. coleopterorum	Coleoptera	Samson 1974; Samson & Evans 1977
I. farinosa	Acari, Aranea, Coleoptera, Chelonethida, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Opiliones, Thysanoptera.	see Zimmermann 2008
I. fumosorosea	Acari, Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, Thysanoptera.	see Zimmermann 2008
I. javanica	Coleoptera, Lepidoptera, Aranaea	Samson 1974; Samson & Evans 1977; Luangsa-ard *et al.* 2005
I. ghanensis	Lepidoptera	Samson 1974; Samson & Evans 1977
I. tenuipes	Coleoptera, Lepidoptera	Mains 1955; Samson 1974; Luangsa-ard 2004

Currently the genus *Isaria* is poorly known in New Zealand. Two species recorded from this country, *I. sulphurea* and *I. felina,* appear to be saprophytic fungi and are not included in the current circumscription of *Isaria.* Among species now included in the genus, *I. cicadae* is the most frequently reported in New Zealand. The species was first recorded in this country by Taylor (1855) and has been known under several synonyms including *Sphaeria basili, Cordyceps sinclairii* and *Isaria sinclairii.* Although *I. farinosa* was first reported (as *Spicaria farinosa*) from New Zealand early last century (Kirk 1909), there have been few subsequent records (see Anon 2001-2009; Glare *et al.* 1993b) and information on the host range of the

species in this country is limited. Other *Isaria* species which are relatively common in other parts of the world have been only rarely collected in this country, with *I. tenuipes*, *I. javanica*, and *I. fumosorosea* each represented by a single New Zealand collection (Anon 2001-2009). This chapter discusses the morphological and molecular characterisation of *Isaria* species collected and isolated from arthropod hosts in native forests.

4.2 Methods

4.2.1 Morphological characterisation

Isaria species were collected and isolated from native forest as described in chapter two. Specimens and corresponding cultures were identified as *Isaria* according to Samson (1974) and Luangsa-ard *et al.* (2005). Material was identified as *Isaria* based on conidiogenous structures *i.e.* verticillately branched conidiophores bearing dense whorls of phialides with a swollen base and distinct neck, producing conidia in chains. For species determination, measurements were made from conidia taken directly from infected hosts and from cultures grown at 25°C for 14 days in darkness on 2% malt extract agar (MEA: 20g/L malt extract, 15g/L agar). For each specimen and culture, length and width of 25 conidia was recorded. Length and width of 25 phialides was measured directly from host material. Methods used to obtain measurements are given in chapter two. Species were determined by comparing phialide and conidial morphology with the descriptions given by Samson (1974). To determine any significant relationship between host affiliation and conidia/philaide size within *Isaria farinosa,* mean conidia and phialide sizes were square-root transformed and analysed by ANOVA. Means were separated by Tukeys test. Statistical analyses were performed using SPSS version 11.00

4.2.2 Molecular characterisation

To confirm species identifications and examine phylogenetic relationships in New Zealand *Isaria,* representative isolates were selected for sequencing based on the range of morphological diversity and host affiliations observed. All methods used for DNA extraction, PCR, sequencing, and phylogenetic analysis are given in Chapter Two.

For initial phylogenetic analysis the entire ITS region was amplified and sequenced using the primers ITS4 and ITS5 (White *et al.* 1990). Sequences were aligned with *Isaria* sequences from Genbank. *Cordyceps cardinalis* and *Cordyceps pseudomilitaris* were used as outgroup taxa based on their basal relationship to *Isaria* species in the phylogeny of Sung *et al.* 2007a. Phylogenetic analysis was conducted using maximum parsimony and Bayesian likelihood inference. To provide further support for phylogenetic groups indicated from the ITS phylogeny, a subset of isolates was selected for analysis of β-tubulin and EF1-α gene regions. Primers and PCR conditions are described in chapter two. For each region sequences were aligned with *Isaria* sequences from Genbank. As few sequences from the Bt2a/Bt2b region for were available, *Paecilomyces lilacinus* and *Cordyceps cylindrica* were selected as the closest usable outgroup taxa for the β-tubulin analysis. *Simplicillium lanosoniveum* and *Simplicillium*

lamellicola were selected as the closest usable outgroup taxa for the EF1-α analysis. Phylogenetic analysis for β-tubulin and EF1-α was conducted as above. Genbank accession numbers and strain details for overseas isolates are shown in Table 4.2.

Table 4.2 Genbank sequences included in phylogenetic analysis of *Isaria* species.

Species	Strain	Country	ITS	β-tubulin	EF1-α	Reference
Isaria amoenerosea	CBS 107.73		AY624168	AY624207		Luangsa-ard *et al.* 2005
Isaria amoenerosea	CBS 729.73	Ghana	AY624169	AY624208		Luangsa-ard *et al.* 2005
Isaria cateniannulata	CBS 152.83	China	AY624172	AY624211		Luangsa-ard *et al.* 2005
Isaria cateniobliqua	CBS 153.83	China	AY624173	AY624212		Luangsa-ard *et al.*2005
Isaria cateniobliqua	RCEF189	China	AF368799			Huang *et al.* unpublished
Isaria cicadae	IFO33061	Japan	AB086630			Yokoyama *et al.* 2004
Isaria cicadae	RCEF200	China	AF368801			Huang et al unpublished
Isaria farinosa	CBS 111113	Denmark	AY624181	AY624219		Luangsa-ard *et al.* 2005
Isaria farinosa	CBS 541.81	Galapagos Is.	AY624180	AY624218		Luangsa-ard *et al.* 2005
Isaria farinosa	CBS 262.58	United Kingdom	AY624179	AY624217		Luangsa-ard *et al.* 2005
Isaria farinosa	CBS 240.32	The Netherlands	AY624178			Luangsa-ard *et al.* 2005
Isaria farinosa	IFO8108	Japan	AB080087			Yokoyama *et al.* 2004
Isaria farinosa	RCEF632	China	AF368788			Huang *et al.* unpublished
Isaria farinosa	OSC111005	United States			EF469065	Sung *et al.* 2007a
Isaria farinosa	OSC 111006	United States			DQ522348	Sung *et al.* 2007a
Isaria cf. farinosa	OSC 111004	United States			EF468780	Sung *et al.* 2007a
Isaria fumosorosea	CBS 375.70	Japan	AY624183	AY624221		Luangsa-ard *et al.* 2005
Isaria fumosorosea	CBS 244.31	Ireland	AY624182	AY624220		Luangsa-ard *et al.* 2005
Isaria ghanensis	CBS 105.73	Ghana	AY624185	AY624223		Luangsa-ard *et al.* 2005
Isaria javanica	CBS 134.22	Indonesia	AY624186			Luangsa-ard *et al.* 2005
Isaria javanica	CM1	China	EF990131			Huang *et al.* unpublished
Isaria tenuipes	ARSEF 5135	North America	AY624196	AY624234		Luangsa-ard *et al.* 2005
Isaria tenuipes	CBS 994.73	The Netherlands	AY624195	AY624233		Luangsa-ard et al. 2005
Isaria tenuipes	BCC 2787	Thailand	AY624200			Luangsa-ard *et al.* 2005
Isaria tenuipes	BCC2918	Thailand			FJ472845	Luangsa-ard & Ridkaew unpublished
Isaria tenuipes	OSC 111007	United States			DQ522349	Sung *et al.* 2007a
Cordyceps cylindrica	BCC2102	Thailand		EF411266		Luangsa-ard *et al.* unpublished

Table 4.2 continued

Species	Strain	Country	ITS	β-tubulin	EF1-α	Reference
Cordyceps cardinalis	BCMU CC01	Japan	AB237660			Yokoyama *et al.* unpublished
Cordyceps pseudomilitaris	NHJ6	Thailand	AJ786589			Stensrud *et al.* 2005
Paecilomyces lilacinus	CBS284.36			AY624227		Luangsa-ard *et al.* 2005
Simplicillium lanosoniveum	CBS 704.86				DQ522358	Spatafora *et al.* 2007
Simplicillium lamellicola	CBS 116.25				DQ522356	Spatafora *et al.* 2007

4. 3 Results

4.3.1 *Isaria* collections

Table 4.3 Total numbers of *Isaria* specimens collected from different host orders and regions.

	Brunner/ Westland	Nelson/Tasman	North Canterbury	Bay of Plenty	Tongariro/ Rangitikei	Total
Acari		1				1
Aranaea	2					2
Coleoptera					1	1
Hemiptera	14	2			14	30
Hymenoptera	1	2			1	4
Lepidoptera	17	5	1	1	12	36
Opiliones		1				1
Unidentified	1	2	1		4	8
Total	35	13	2	1	32	83

Table 4.4 Host associations of *Isaria* species.

	I. farinosa	*I. cf. farinosa*	*I. tenuipes*	*I. cicadae*	*I. cf. cicadae*
Acari	1				
Aranaea	2				
Coleoptera					1
Hemiptera	1			29	
Hymenoptera	4				
Lepidoptera	17	5	12		2
Opiliones	1				
Unidentified	8				
Total	34	5	12	29	3

Table 4.5 Regional collections of *Isaria* species.

	I. farinosa	*I. cf. farinosa*	*I. tenuipes*	*I. cicadae*	*I. cf. cicadae*
Brunner/Westland	6	4	11	14	
Nelson/Tasman	12			1	
North Canterbury	2				
Bay of Plenty			1		
Tongariro/Rangitikei	14	1		14	3

Table 4.6 *Isaria* specimens collected and examined in this study. Specimen numbers include collection date as *yy/mm/dd.x*.

Isolate #	Specimen #	Locality	Species	Host	Host stage	Region
E1044	040513.8	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	Acari	adult	Nelson/Tasman
E1094	040610.1	Lake Kaniere Walkway, Hokitika	*I. farinosa*	Araneae	adult	Brunner/Westland
NC177	060415.39	Lake Kaniere Walkway, Hokitika	*I. farinosa*	Araneae	adult	Brunner/Westland
NC122	050405.14	Paengaroa Scenic Reserve, Mataroa	*I. cf. cicadae*	Coleoptera	adult	Tongariro/Rangitikei
NC221	060508.7	Charming Creek Walkway, Westport	*I. cicadae*	Hemiptera: Cicadidae	nymph	Nelson/Tasman
NC20	050406.11	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC22	050406.13	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC24	050406.15	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC25	050406.16	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC26	050406.17	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC27	050406.18	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC29	050406.20	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC30	050406.21	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC31	050406.22	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC33	050406.24	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC34	050406.25	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC35	050408.15	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC121	050408.9	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC128	050408.16	Mangawhero Forest Walk, Mount Ruapehu	*I. cicadae*	Hemiptera: Cicadidae	nymph	Tongariro/Rangitikei
NC7	050302.2	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC8	050302.3	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC9	050302.4	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC10	050302.5	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC12	050302.6	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC14	050302.10	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland

Table 4.6 continued.

Isolate #	Specimen #	Locality	Species	Host	Host stage	Region
NC15	050302.11	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC16	050302.12	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC17	050302.13	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC18	050302.14	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC19	050302.15	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC37	050302.8	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC38	050302.9	Pororari River Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
NC6	050302.1	Truman Track, Punakaiki	*I. cicadae*	Hemiptera: Cicadidae	nymph	Brunner/Westland
E1051	040510.6	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	Hemiptera	adult	Nelson/Tasman
TE443	020417.10	Lake Kaniere Walkway, Hokitika	*I. farinosa*	Hymenoptera	adult	Brunner/Westland
NC126	050408.17	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Hymenoptera	adult	Tongariro/Rangitikei
E1048	040514.3	Loop Track, Lake Rotoiti	*I. farinosa*	Hymenoptera: Vespidae	adult	Nelson/Tasman
E1054	040513.7	Rolling Creek, Wangapeka Valley	*I. farinosa*	Hymenoptera: Vespidae	adult	Nelson/Tasman
NC112	050406.7	Mangawhero Forest Walk, Mount Ruapehu	*I. cf. cicadae*	Lepidoptera	pupa	Tongariro/Rangitikei
NC127	050406.35	Mangawhero Forest Walk, Mount Ruapehu	*I. cf. cicadae*	Lepidoptera	pupa	Tongariro/Rangitikei
TE02	010420.1	Lake Kaniere Walkway, Hokitika	*I. farinosa*	Lepidoptera	larva	Brunner/Westland
NC76	050302.16	Truman Track, Punakaiki	*I. farinosa*	Lepidoptera	adult	Brunner/Westland
NC117	050408.25	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	larva	Tongariro/Rangitikei
NC124	050408.13	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	larva	Tongariro/Rangitikei
NC131	050408.7	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	larva	Tongariro/Rangitikei
NC63	050405.11	Paengaroa Scenic Reserve, Mataroa	*I. farinosa*	Lepidoptera	larva	Tongariro/Rangitikei
E1049	040510.14	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	Lepidoptera	pupa	Nelson/Tasman
E1056	040510.13	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	Lepidoptera	pupa	Nelson/Tasman
E1053	040514.7	Loop Track, Lake Rotoiti	*I. farinosa*	Lepidoptera	pupa	Nelson/Tasman
NC203	060508.5	Charming Creek Walkway, Westport	*I. farinosa*	Lepidoptera	pupa	Nelson/Tasman
NC94	050510.7	Devils Punchbowl Track, Arthurs Pass	*I. farinosa*	Lepidoptera	pupa	North Canterbury

Table 4.6 continued.

Isolate #	Specimen #	Locality	Species	Host	Host stage	Region
NC125	050404.18	Mangawhero Falls Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
NC55	050408.8	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
NC56	050408.11	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
NC116	050408.21	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
NC129	050408.19	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
NC206	060509.2	Nikau Loop Walk, Karamea	*I. farinosa*	Lepidoptera	pupa	Brunner/Westland
NC181	060415.42	Lake Kaniere Walkway, Hokitika	*I. cf. farinosa*	Lepidoptera	pupa	Brunner/Westland
NC184	060415.46	Lake Kaniere Walkway, Hokitika	*I. cf. farinosa*	Lepidoptera	pupa	Brunner/Westland
NC180	060415.41	Lake Kaniere Walkway, Hokitika	*I. cf. farinosa*	Lepidoptera	pupa	Brunner/Westland
NC212	060511.10	Nile River Valley Walk, Charleston	*I. cf. farinosa*	Lepidoptera	pupa	Brunner/Westland
NC108	050405.12	Paengaroa Scenic Reserve, Mataroa	*I. cf. farinosa*	Lepidoptera	pupa	Tongariro/Rangitikei
E378	030506.6	Lindemann Loop Track, Katikati	*I. tenuipes*	Lepidoptera	pupa	Bay of Plenty
TE677	020507.3	Cascade Valley, Haast	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
TE433	020417.0	Goldsborough (Shamrock) Track, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
TE434	020417.1	Goldsborough (Shamrock) Track, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
TE497	020509.1	Hapuka Estuary Walk, Okuru	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
E1095	040610.2	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
E1096	040610.3	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
NC182	060415.45	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
TE435	020417.2	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
TE436	020417.3	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
E090	030421.1	Lake Kaniere Walkway, Hokitika	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
NC200	060508.1	Nile River Valley Walk, Charleston	*I. tenuipes*	Lepidoptera	pupa	Brunner/Westland
E1045	040510.21	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	Opiliones	adult	Nelson/Tasman
NC80	050215.3	Devils Punchbowl Track, Arthurs Pass	*I. farinosa*	n.d.	n.d	North Canterbury

Table 4.6 continued.

Isolate #	Specimen #	Locality	Species	Host	Host stage	Region
TE437	020417.4	Lake Kaniere Walkway, Hokitika	*I. farinosa*	n.d.	n.d.	Brunner/Westland
E1046	040510.4	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	n.d.	n.d.	Nelson/Tasman
E1047	040510.20	Snowdens Bush Scenic Reserve, Brightwater	*I. farinosa*	n.d.	n.d.	Nelson/Tasman
NC54	050404.26	Mangawhero Falls Walk, Mount Ruapehu	*I. farinosa*	n.d.	n.d	Tongariro/Rangitikei
NC113	050406.8	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	n.d.	n.d.	Tongariro/Rangitikei
NC118	050406.27	Mangawhero Forest Walk, Mount Ruapehu	*I. farinosa*	n.d	n.d.	Tongariro/Rangitikei
NC61	050405.5	Paengaroa Scenic Reserve, Mataroa	*I. farinosa*	n.d.	n.d.	Tongariro/Rangitikei

n.d. not determined.

4.3.2 Morphological characterisation

Isaria farinosa

Conidia from *I. farinosa* were ellipsoidal to fusiform (Fig. 4.2A) and measured 2.0-2.9 × 1.1-1.9 µm (average 2.4 × 1.4 µm) on the host. Conidia from cultures on MEA after 14 days measured 2.1-3.2 × 1.1-2.0 µm (average 2.6 × 1.5 µm). Phialides from the host (Fig. 4.2C-E) measured 3.8-9.8 × 1.9-3.5 µm (average 5.8 × 2.5 µm). Conidia and phialide sizes from different host orders are shown in Table 4.7. Conidia and phialides were significantly longer on non-insect (arachnid) hosts.

Table 4.7 Conidia and phialide sizes of *I. farinosa* from different host orders.

	Conidia on host length × width	Conidia on MEA length × width	Phialides on host length × width
Acari	2.1-2.6 (2.4) × 1.1-1.5(1.3)	2.5-3.1 (2.7) × 1.1-1.7 (1.3)	4.5-7.3 (6.0) × 2.4-3.1 (2.8)
Aranaea	2.3-2.9 (2.7) × 1.3-1.8 (1.6)	2.3-3.0 (2.7) × 1.2-1.9 (1.6)	4.5-7.9 (6.1) × 2.0-2.9 (2.5)
Hemiptera	2.3-2.6 (2.4) × 1.2-1.7 (1.5)	2.3-3.0 (2.6) × 1.3-1.8 (1.5)	3.9-7.0 (5.5) × 1.9-2.7 (2.2)
Hymenoptera	2.1-2.9 (2.4) × 1.1-1.7 (1.4)	2.1-3.2 (2.6) × 1.2-1.9 (1.4)	4.1-7.2 (5.9) × 1.9-3.0 (2.5)
Lepidoptera	2.0-2.9 (2.4) × 1.1-1.9 (1.4)	2.1-3.1 (2.6) × 1.1-2.0 (1.5)	3.8-8.3 (5.6) × 2.0-3.5 (2.5)
Opiliones	2.3-2.9 (2.6) × 1.4-1.7 (1.6)	2.3-3.1 (2.7) × 1.1-1.7 (1.4)	5.4-9.8 (7.3) × 2.4-3.3 (2.9)
Unidentified	2.0-2.9 (2.4) × 1.1-1.7 (1.4)	2.1-3.1 (2.6) × 1.1-1.9 (1.4)	4.1-8.7(5.8) ×.1.9-3.2(2.5)

Isaria cf. farinosa

Conidia were ellipsoidal to fusiform (Fig 4.2B) and measured 2.5-3.8 × 1.5-2.5 µm (average 3.1 × 1.9 µm) on the host. Conidia from cultures on MEA after 14 days measured 2.6-4.0 × 1.8-2.7 µm (average 3.2 × 2.0 µm). Phialides from the host (Fig 4.2F-H) measured 4.6-11.4 × 2.3-4.0 (average 6.6 × 3.1 µm).

Isaria tenuipes

Conidia were ellipsoidal to cylindrical, often slightly curved or allantoid (Fig 4.4A-C). Conidia measured 3.0-6.9 × 1.2-2.4 µm (average 4.7 × 1.7 µm) on the host. Conidia from cultures on MEA after 14 days measured 3.9-9.1 × 1.4-3.5 µm (average 6.0 × 2.0 µm). Phialides from the host (Fig. 4.4D-H) measured 3.6-7.2 × 2.5-5.3 µm (average 5.0 × 3.5 µm).

Isaria cicadae

Conidia were cylindrical, usually highly curved (Fig. 4.6A), and measured 3.2-6.5 × 1.1-2.7 μm (average 4.2 × 1.5 μm) on the host. Conidia from cultures on MEA after 14 days measured 3.7-12.3 × 1.4-2.7 μm (average 7.1 × 2.0 μm). Phialides from the host (Fig. 4.6C-D) measured 3.5-6.6 × 2.4-5.1 μm (average 4.9 × 3.4 μm).

Isaria cf. cicadae

Conidia were cylindrical, curved (Fig. 4.6B), and measured 5.7-9.0 × 1.6-2.5 μm (average 7.0 × 2.0 μm) on the host. Conidia from cultures on MEA after 14 days measured 6.2-13.7 × 1.4-3.0 μm (average 9.2 × 2.1 μm). Phialides from the host (Fig. 4.6E-F) measured 4.4-6.9 × 3.2-5.4 μm (average 5.7 × 4.2 μm).

Table 4.8 Measurements of conidia from host material and 14 day old MEA cultures, and phialide measurements from host material. All measurements are given in µm with averages (n=25) in brackets.

Isolate #	Specimen #	Species	Host	Conidia on MEA length × width	Conidia on host length × width	Phialides on host length × width
E1044	040513.8	*I. farinosa*	Acari	2.5-3.1 (2.7) × 1.1-1.7 (1.3)	2.1-2.6 (2.4) × 1.1-1.5(1.3)	4.5-7.3 (6.0) × 2.4-3.1 (2.8)
E1094	040610.1	*I. farinosa*	Araneae	2.3-3.0 (2.7) × 1.2-1.9 (1.5)	2.3-2.8 (2.6) × 1.3-1.7 (1.5)	4.7-7.9 (6.5) × 2.4-2.9 (2.6)
NC177	060415.39	*I. farinosa*	Araneae	2.3-3.0 (2.6) × 1.4-1.9 (1.6)	2.3-2.9 (2.7) × 1.4-1.8 (1.6)	4.5-7.0 (5.7) × 2.0-2.7 (2.3)
NC122	050405.14	*I. cf. cicadae*	Coleoptera	6.2-10.9 (8.0) × 1.4-2.2 (1.8)	6.0-7.9 (6.8) × 1.6-2.5 (1.9)	4.4-6.9 (5.8) × 3.2-5.4 (4.2)
NC221	060508.7	*I. cicadae*	Hemiptera	3.7-10.8 (6.1) × 1.4-2.5 (1.8)	3.8-5.4 (4.5) × 1.4-1.9 (1.6)	3.7-6.4 (4.8) × 3.0-4.2 (4.8)
NC20	050406.11	*I. cicadae*	Hemiptera	4.1-10.0 (6.4) × 1.5-2.4 (1.9)	3.5-5.5 (4.3) × 1.2-1.7 (1.4)	4.0-5.6 (4.8) × 2.5-4.0 (3.0)
NC22	050406.13	*I. cicadae*	Hemiptera	4.0-11.9 (7.0) × 1.5-2.6 (2.1)	3.4-4.6 (3.8) × 1.3-1.7 (1.4)	4.2-6.1 (5.1) × 2.7-3.6 (3.3)
NC24	050406.15	*I. cicadae*	Hemiptera	4.0-10.9 (6.4) × 1.5-2.5 (2.0)	3.2-5.0 (4.0) × 1.2-2.0 (1.4)	4.2-6.1 (5.1) × 3.2-4.1 (3.6)
NC25	050406.16	*I. cicadae*	Hemiptera	3.8-9.7 (6.6) × 1.7-2.6 (2.1)	3.2-4.5 (4.0) × 1.1-1.7 (1.4)	3.5-5.3 (4.6) × 3.1-3.7 (3.3)
NC26	050406.17	*I. cicadae*	Hemiptera	3.8-11.5 (7.3) × 1.6-2.6 (2.1)	3.5-5.0 (4.3) × 1.4-1.7 (1.6)	4.0-6.0 (4.9) × 3.0-4.1 (3.4)
NC27	050406.18	*I. cicadae*	Hemiptera	4.1-11.4 (7.0) × 1.5-2.3 (2.0)	3.5-4.9 (4.0) × 1.2-1.8 (1.4)	3.7-5.8 (4.7) × 2.9-3.6 (3.1)
NC29	050406.20	*I. cicadae*	Hemiptera	4.2-11.2 (6.4) × 1.5-2.7 (2.1)	3.5-5.1 (4.2) × 1.2-1.8 (1.4)	4.4-5.8 (5.2) × 2.7-4.0 (3.5)
NC30	050406.21	*I. cicadae*	Hemiptera	3.7-9.4 (6.6) × 1.7-2.4 (2.1)	3.7-4.7 (4.1) × 1.4-1.8 (1.6)	4.1-6.1 (4.8) × 2.7-3.8 (3.4)
NC31	050406.22	*I. cicadae*	Hemiptera	3.8-10.8 (7.2) × 1.5-2.4 (1.9)	3.5-4.9 (4.1) × 1.3-1.8 (1.5)	3.7-5.4 (4.6) × 2.8-3.9 (3.4)
NC33	050406.24	*I. cicadae*	Hemiptera	3.9-11.4 (6.7) × 1.5-2.3 (1.8)	3.4-4.2 (3.7) × 1.2-1.6 (1.4)	4.1-6.0 (4.8) × 2.7-3.5 (3.1)
NC34	050406.25	*I. cicadae*	Hemiptera	4.1-11.9 (7.3) × 1.5-2.5 (2.1)	3.2-5.9 (4.0) × 1.4-1.9 (1.6)	4.2-5.5 (4.8) × 2.9-3.7 (3.3)
NC35	050408.15	*I. cicadae*	Hemiptera	4.0-10.0 (7.1) × 1.5-2.5 (2.0)	3.7-5.0 (4.3) × 1.4-1.8 (1.6)	4.1-5.3 (4.6) × 2.9-3.4 (3.1)
NC121	050408.9	*I. cicadae*	Hemiptera	3.9-11.5 (7.1) × 1.6-2.5 (2.0)	3.7-5.7 (4.3) × 1.4-2.1 (1.7)	4.1-5.7 (4.9) × 3.4- 4.5 (3.9)
NC128	050408.16	*I. cicadae*	Hemiptera	3.9-12.3 (7.0) × 1.5-2.5 (1.9)	3.5-5.0 (4.1) × 1.3-1.7 (1.5)	4.0-6.0 (5.2) × 2.7-4.0 (3.4)
NC7	050302.2	*I. cicadae*	Hemiptera	4.0-11.2 (6.6) × 1.5-2.5 (1.9)	3.4-4.5 (3.7) × 1.1-1.6 (1.4)	3.8-6.0 (4.8) × 2.5-4.2 (3.2)
NC8	050302.3	*I. cicadae*	Hemiptera	3.9-11.9 (8.2) × 1.4-2.5 (2.1)	3.7-5.1 (4.2) × 1.4-1.9 (1.6)	4.4-6.4 (5.1) × 2.7-3.7 (3.1)
NC9	050302.4	*I. cicadae*	Hemiptera	4.1-11.9 (7.8) × 1.5-2.6 (2.1)	3.8-6.5 (4.8) × 1.6-2.7 (2.0)	3.7-5.4 (4.5) × 2.4-3.6 (3.0)
NC10	050302.5	*I. cicadae*	Hemiptera	4.1-11.5 (7.1) × 1.6-2.6 (2.1)	3.5-5.0 (4.2) × 1.3-1.7 (1.5)	4.1-6.3 (5.0) × 3.1- 5.1 (3.8)
NC12	050302.6	*I. cicadae*	Hemiptera	4.2-12.1 (8.7) × 1.5-2.5 (2.0)	3.5-5.5 (4.4) × 1.2-1.8 (1.5)	4.0-6.6 (5.0) × 3.1-4.0 (3.6)

Table 4.8. continued.

Isolate #	Specimen #	Species	Host	Conidia on MEA length × width	Conidia on host length × width	Phialides on host length × width
NC14	050302.10	*I. cicadae*	Hemiptera	4.5-10.3 (7.4) × 1.7-2.5 (2.1)	3.6-5.3 (4.4) × 1.2-1.7 (1.4)	4.2-5.9 (5.1) × 2.9-4.2 (3.6)
NC15	050302.11	*I. cicadae*	Hemiptera	3.8-11.8 (7.5) × 1.5-2.6 (2.0)	3.6-5.4 (4.4) × 1.1-1.8 (1.5)	4.2-5.8 (5.0) × 2.9-4.1 (3.6)
NC16	050302.12	*I. cicadae*	Hemiptera	3.5-11.3 (8.1) × 1.4-2.6 (2.0)	3.6-4.7 (4.1) × 1.2-1.7 (1.5)	4.1-6.1 (5.1) × 3.2-4.1 (3.6)
NC17	050302.13	*I. cicadae*	Hemiptera	3.9-11.3 (6.9) × 1.5-2.3 (2.0)	3.3-5.5 (4.2) × 1.4-1.8 (1.6)	4.2-5.8 (5.1) × 2.9-4.9 (3.6)
NC18	050302.14	*I. cicadae*	Hemiptera	3.9-10.3 (6.6) × 1.6-2.9 (2.2)	3.2-4.7 (3.8) × 1.3-1.9 (1.6)	3.9-5.8 (4.8) × 3.0-4.1 (3.5)
NC19	050302.15	*I. cicadae*	Hemiptera	3.9-10.7 (7.2) × 1.3-2.2 (1.9)	3.3-6.0 (4.3) × 1.3-1.9 (1.7)	4.4-6.4 (5.2) × 3.0-4.2 (3.5)
NC37	050302.8	*I. cicadae*	Hemiptera	3.6-10.3 (6.7) × 1.5-2.6 (2.0)	3.7-4.8 (4.2) × 1.3-1.8 (1.6)	3.9-6.1 (4.5) × 2.7-3.6 (3.3)
NC38	050302.9	*I. cicadae*	Hemiptera	3.8-10.1 (7.5) × 1.5-2.2 (1.8)	3.6-5.1 (4.3) × 1.2-1.7 (1.5)	4.0-5.1 (4.6) × 3.0-4.2 (3.5)
NC6	050302.1	*I. cicadae*	Hemiptera	4.2-11.2 (8.0) × 1.4-2.5 (2.0)	3.5-5.3 (4.3) × 1.2-1.8 (1.5)	3.9-5.6 (4.7) × 2.6-3.8 (3.2)
E1051	040510.6	*I. farinosa*	Hemiptera	2.3-3.0 (2.6) × 1.3-1.8 (1.5)	2.3-2.6 (2.4) × 1.2-1.7 (1.5)	3.9-7.0 (5.5) × 1.9-2.7 (2.2)
TE443	020417.10	*I. farinosa*	Hymenoptera	2.2-2.9 (2.6) × 1.2-1.7 (1.5)	2.2-2.9 (2.5) × 1.2-1.7 (1.4)	5.5-7.1 (6.4) × 1.9-2.7 (2.3)
NC126	050408.17	*I. farinosa*	Hymenoptera	2.4-2.9 (2.7) × 1.2-1.6 (1.4)	2.1-2.5 (2.3) × 1.2-1.6 (1.4)	4.1-7.0 (5.3) × 2.0-2.8 (2.5)
E1048	040514.3	*I. farinosa*	Hymenoptera	2.3-3.2 (2.7) × 1.2-1.9 (1.4)	2.3-2.8 (2.5) × 1.1-1.7 (1.4)	5.6-7.2 (6.3) × 2.4-3.0 (2.6)
E1054	040513.7	*I. farinosa*	Hymenoptera	2.1-3.0 (2.5) × 1.2-1.6 (1.4)	2.2-2.7 (2.4) × 1.2-1.6 (1.4)	4.5-7.1 (5.6) × 2.3-3.0 (2.6)
NC112	050406.7	*I. cf. cicadae*	Lepidoptera	6.9-11.8 (9.4) × 1.7-2.9 (2.1)	5.7-7.8 (6.6) × 1.8-2.2 (2.0)	4.6-6.6 (5.6) × 3.4-4.8 (4.1)
NC127	050406.35	*I. cf. cicadae*	Lepidoptera	7.2-13.7 (10.3) × 1.7-3.0 (2.3)	6.1-9.0 (7.7) × 1.7-2.5 (2.1)	5.1-6.7 (5.7) × 3.6-4.8 (4.2)
NC76	050302.16	*I. farinosa*	Lepidoptera	2.3-2.9 (2.6) × 1.1-1.4 (1.2)	2.1-2.7 (2.4) × 1.2-1.7 (1.4)	4.7-7.6 (6.0) × 2.2-3.0 (2.6)
TE02	010420.1	*I. farinosa*	Lepidoptera	2.3-2.1(2.8) × 1.2-1.6 (1.4)	2.1-2.8 (2.3) × 1.1-1.6 (1.3)	4.2-6.7 (5.6) × 2.3-3.4 (2.9)
NC117	050408.25	*I. farinosa*	Lepidoptera	2.2-2.8 (2.5) × 1.3-1.7 (1.5)	2.2-2.6 (2.4) × 1.3-1.7 (1.5)	4.6-7.1 (5.6) × 2.1-3.0 (2.6)
NC124	050408.13	*I. farinosa*	Lepidoptera	2.1-2.8 (2.4) × 1.2-1.7 (1.5)	2.1-2.6 (2.3) × 1.1-1.6 (1.4)	4.3-6.4 (5.4) × 2.2-2.8 (2.5)
NC131	050408.7	*I. farinosa*	Lepidoptera	2.4-3.0 (2.7) × 1.4-2.0 (1.7)	2.0-2.5 (2.3) × 1.1-1.5 (1.3)	4.5-6.4 (5.4) × 2.0-2.8 (2.4)
NC63	050405.11	*I. farinosa*	Lepidoptera	2.1-2.8 (2.4) × 1.1-1.6 (1.4)	2.0-2.7 (2.3) × 1.2-1.6 (1.4)	4.3-6.9 (5.4) × 2.3-2.9 (2.5)
E1049	040510.14	*I. farinosa*	Lepidoptera	2.2-2.9 (2.6) × 1.2-1.6 (1.4)	2.0-2.5 (2.3) × 1.2-1.7 (1.4)	4.5-7.9 (5.8) × 2.1-3.1 (2.6)
E1056	040510.13	*I. farinosa*	Lepidoptera	2.2-3.1 (2.6) × 1.2-1.7 (1.5)	2.1-2.6 (2.3) × 1.2-1.6 (1.4)	5.9-7.9 (6.6) × 2.5-3.2 (2.8)
E1053	*040514.7*	*I. farinosa*	Lepidoptera	2.3-2.9 (2.7) × 1.2-1.8 (1.5)	2.2-2.7 (2.4) × 1.3-1.6 (1.5)	4.8-8.3 (5.8) × 2.4-3.3 (2.8)

Table 4.8. continued.

Isolate #	Specimen #	Species	Host	Conidia on MEA length × width	Conidia on host length × width	Phialides on host length × width
NC203	060508.5	*I. farinosa*	Lepidoptera	2.3-3.1 (2.6) × 1.3-1.7 (1.5)	2.2-2.8 (2.4) × 1.3-1.7 (1.5)	4.7-6.9 (5.5) × 2.5-3.5 (2.9)
NC94	050510.7	*I. farinosa*	Lepidoptera	2.5-2.9 (2.7) × 1.2-1.7 (1.5)	2.1-2.6 (2.3) × 1.2-1.6 (1.4)	3.8-7.2 (5.4) × 2.0-2.9 (2.3)
NC125	050404.18	*I. farinosa*	Lepidoptera	2.1-2.6 (2.4) × 1.2-1.7 (1.5)	2.1-2.7 (2.4) × 1.2-1.5 (1.3)	4.1-6.9 (5.5) × 2.1-2.9 (2.5)
NC55	050408.8	*I. farinosa*	Lepidoptera	2.1-2.8 (2.5) × 1.2-1.7 (1.5)	2.1-2.7 (2.4) × 1.1-1.6 (1.4)	4.4-6.9 (5.7) × 2.0-3.1 (2.4)
NC56	050408.11	*I. farinosa*	Lepidoptera	2.3-3.0 (2.6) × 1.2-1.7 (1.4)	2.2-2.7 (2.4) × 1.4-1.8 (1.6)	4.7-7.1 (5.9) × 2.2-3.2 (2.5)
NC116	050408.21	*I. farinosa*	Lepidoptera	2.2-2.8 (2.5) × 1.2-1.7 (1.5)	2.2-2.7 (2.4) × 1.2-1.7 (1.5)	4.2-6.5 (5.6) × 2.2-2.9 (2.5)
NC129	050408.19	*I. farinosa*	Lepidoptera	2.5-3.1 (2.8) × 1.2-1.7 (1.4)	2.2-2.9 (2.6) × 1.2-1.6 (1.4)	4.2-6.9 (5.8) × 2.0-2.9 (2.3)
NC206	060509.2	*I. farinosa*	Lepidoptera	2.4-3.0 (2.7) × 1.5-1.9 (1.6)	2.2-2.9 (2.6) × 1.2-1.9 (1.5)	3.8-7.2 (5.4) × 2.0-2.9 (2.3)
NC181	060415.42	*I. cf. farinosa*	Lepidoptera	2.8-3.9 (3.2) × 1.8-2.5 (2.1)	2.6-3.6 (3.0) × 1.6-2.2 (1.9)	4.9-8.1 (6.3) × 2.6-4.0 (3.3)
NC184	060415.46	*I. cf. farinosa*	Lepidoptera	2.6-3.6 (3.2) × 1.8-2.3 (2.0)	2.5-3.5 (2.9) × 1.8-2.3 (2.0)	5.1-8.5 (6.6) × 2.3-3.1 (2.8)
NC180	060415.41	*I. cf. farinosa*	Lepidoptera	2.7-3.8 (3.2) × 1.8-2.7 (2.1)	2.7-3.5 (3.2) × 1.5-2.0 (1.7)	4.7-7.6 (5.9) × 2.4-3.2 (2.9)
NC212	060511.10	*I. cf. farinosa*	Lepidoptera	2.9-4.0 (3.3) × 1.7-2.5 (2.0)	2.5-3.8 (3.1) × 1.7-2.5 (2.1)	4.6-8.6 (6.7) × 2.9-4.0 (3.4)
NC108	050405.12	*I. cf. farinosa*	Lepidoptera	2.8-3.6 (3.3) × 1.8-2.4 (2.0)	2.7-3.5 (3.1) × 1.8-2.3 (2.0)	5.4-11.4 (7.6) × 2.7-3.8 (3.4)
E378	030506.6	*I. tenuipes*	Lepidoptera	4.8-7.7 (6.1) × 2.1-3.2 (2.5)	3.8-5.2 (4.4) × 1.3-1-8 (1.5)	3.6-5.8 (4.7) × 2.7-3.8 (3.4)
TE677	020507.3	*I. tenuipes*	Lepidoptera	4.6-7.8 (5.7) × 1.9-2.7 (2.2)	3.2-5.1 (3.9) × 1.3-1.8 (1.5)	4.0-6.5 (5.1) × 3.0-4.0 (3.5)
TE433	020417.0	*I. tenuipes*	Lepidoptera	5.1-8.0 (6.4) × 1.5-2.5 (1.9)	4.0-5.4 (4.5) × 1.4-1.8 (1.6)	4.4-6.2 (5.3) × 3.0-4.1 (3.5)
TE434	020417.1	*I. tenuipes*	Lepidoptera	4.0-8.3 (6.1) × 1.4-2.3 (1.9)	3.7-5.3 (4.3) × 1.5-1.9 (1.7)	4.0-6.6 (5.0) × 3.0-5.0 (3.4)
TE497	020509.1	*I. tenuipes*	Lepidoptera	4.2-8.5 (5.7) × 1.4-2.5 (1.9)	4.0-5.3 (4.5) × 1.4-1.9 (1.6)	4.0-5.9 (4.8) × 2.8-4.0 (3.3)
E1095	040610.2	*I. tenuipes*	Lepidoptera	3.9-8.0 (5.4) × 2.0-3.1 (2.3)	4.0-6.2 (5.3) × 1.2-1.9 (1.6)	4.4-6.1 (5.1) × 2.5-3.6 (3.2)
E1096	040610.3	*I. tenuipes*	Lepidoptera	4.2-7.9 (5.6) × 1.9-3.5 (2.3)	4.1-6.6 (5.4) × 1.2-1.9 (1.6)	4.0-6.5 (5.2) × 3.0-4.0 (3.4)
NC182	060415.45	*I. tenuipes*	Lepidoptera	4.5-8.1 (6.1) × 1.7-2.9 (2.3)	4.0-6.9 (5.6) × 1.5-2.3 (1.9)	4.7-7.2 (5.7) × 3.5-5.3 (4.1)
TE435	020417.2	*I. tenuipes*	Lepidoptera	4.6-9.1 (5.9) × 1.9-2.5 (2.1)	4.7-6.6 (5.5) × 1.5-2.0 (1.7)	3.9-5.8 (4.8) × 2.9-3.9 (3.4)
TE436	020417.3	*I. tenuipes*	Lepidoptera	4.5-8.0 (6.1) × 1.4-2.3 (1.8)	3.5-5.0 (4.2) × 1.4-1.9 (1.6)	4.1-6.0 (4.9) × 3.1-3.8 (3.5)
E090	030421.1	*I. tenuipes*	Lepidoptera	4.9-9.0 (6.7) × 1.9-3.0 (2.4)	3.0-4.9 (4.2) × 1.3-1.9 (1.5)	4.3-5.9 (5.3) × 3.0-4.4 (3.7)
NC200	060508.1	*I. tenuipes*	Lepidoptera	4.4-7.7 (6.2) × 2.0-3.2 (2.6)	3.7-5.3 (4.5) × 1.7-2.4 (2.0)	4.0-5.5 (4.5) × 2.8-4.2 (3.5)

Table 4.8. continued.

Isolate #	Specimen #	Species	Host	Conidia on MEA length × width	Conidia on host length × width	Phialides on host length × width
E1045	040510.21	*I. farinosa*	Opiliones	2.3-3.1 (2.7) × 1.1-1.7 (1.4)	2.3-2.9 (2.6) × 1.4-1.7 (1.6)	5.4-9.8 (7.3) × 2.4-3.3 (2.9)
NC80	050215.3	*I. farinosa*	n.d.	2.3-3.1 (2.6) × 1.3-1.6 (1.4)	2.1-2.8 (2.4) × 1.1-1.6 (1.4)	4.2-7.7 (5.8) × 2.0-2.9 (2.4)
TE437	020417.4	*I. farinosa*	n.d.	2.2-3.0 (2.5) × 1.1-1.5 (1.3)	2.2-2.6 (2.4) × 1.2-1.7 (1.5)	4.2-7.1 (5.3) × 2.1-3.0 (2.6)
E1046	040510.4	*I. farinosa*	n.d.	2.2-3.1 (2.7) × 1.3-1.9 (1.5)	2.1-2.8 (2.4) × 1.2-1.6 (1.4)	4.5-8.7 (6.3) × 1.9-3.2 (2.5)
E1047	040510.20	*I. farinosa*	n.d.	2.3-3.1 (2.7) × 1.2-1.9 (1.5)	2.3-2.9 (2.5) × 1.3-1.7 (1.5)	5.1-7.2 (6.2) × 2.3-3.0 (2.6)
NC54	050404.26	*I. farinosa*	n.d.	2.1-2.8 (2.5) × 1.3-1.7 (1.5)	2.0-2.6 (2.3) × 1.2-1.6 (1.4)	4.3-7.2 (5.4) × 2.3-2.8 (2.5)
NC113	050406.8	*I. farinosa*	n.d.	2.1-2.8 (2.4) × 1.2-1.7 (1.4)	2.1-2.6 (2.4) × 1.3-1.7 (1.5)	4.7-7.4 (5.7) × 2.2-2.9 (2.7)
NC118	050406.27	*I. farinosa*	n.d	2.2-2.9 (2.5) × 1.2-1.7 (1.4)	2.1-2.9 (2.4) × 1.2-1.7 (1.3)	4.1-7.0 (5.8) × 2.0-2.9 (2.5)
NC61	050405.5	*I. farinosa*	n.d.	2.3-3.0 (2.6) × 1.3-1.7 (1.5)	2.1-2.8 (2.3) × 1.2-1.6 (1.4)	5.2-6.9 (6.0) × 2.0-2.6 (2.3)

n.d., not determined.

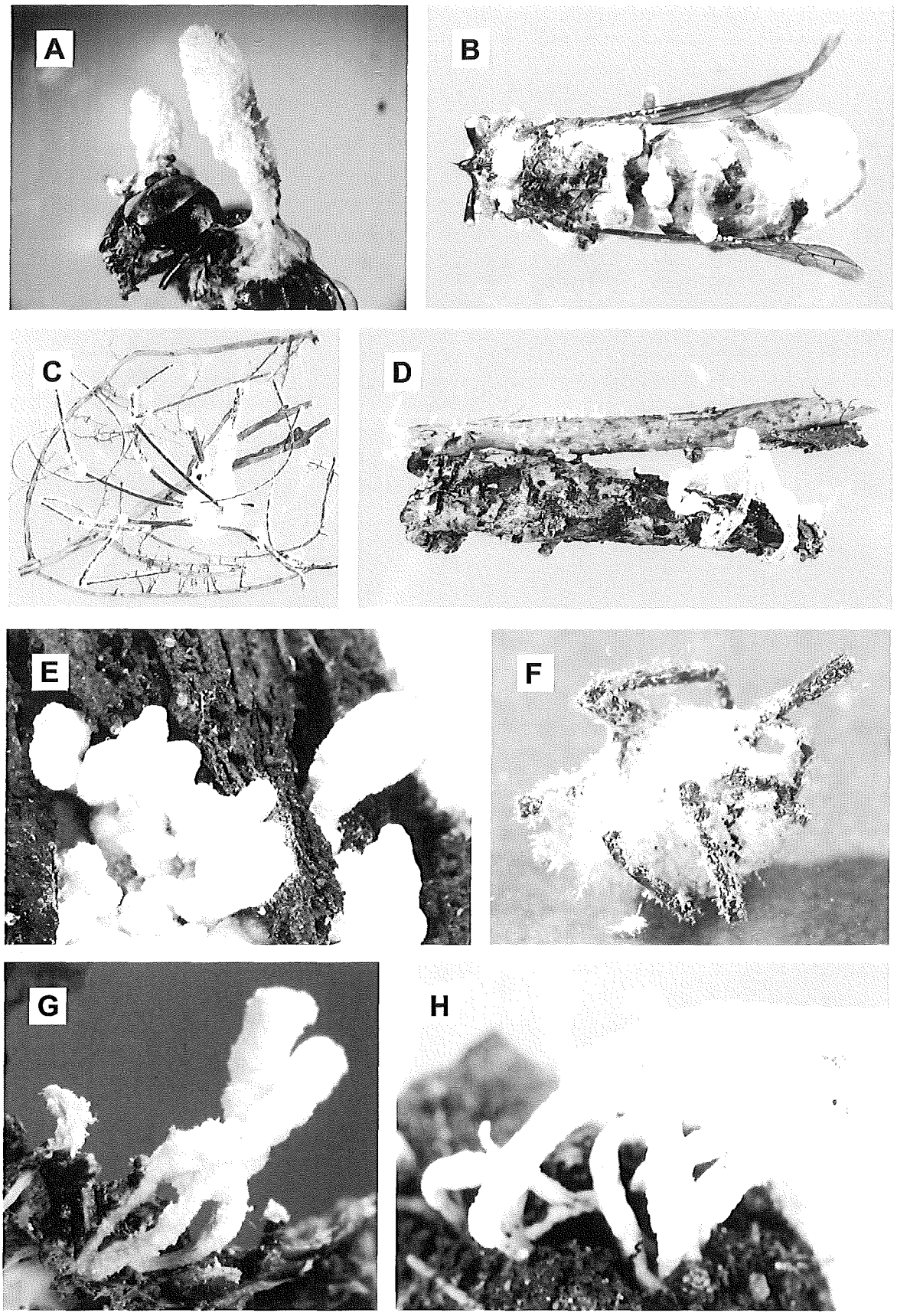

Figure 4.1. A-G *Isaria farinosa*: **A** 050408.17, **B** 050413.7, on Hymenoptera; **C** 040510.21 on Opiliones; **D** 050408.25, **E** 050408.21 on Lepidoptera; **F** 060415.39, on Aranaea; **G** 060508.5, on Lepidoptera. **H:** *Isaria cf. farinosa*, 060415.36 on Lepidoptera.

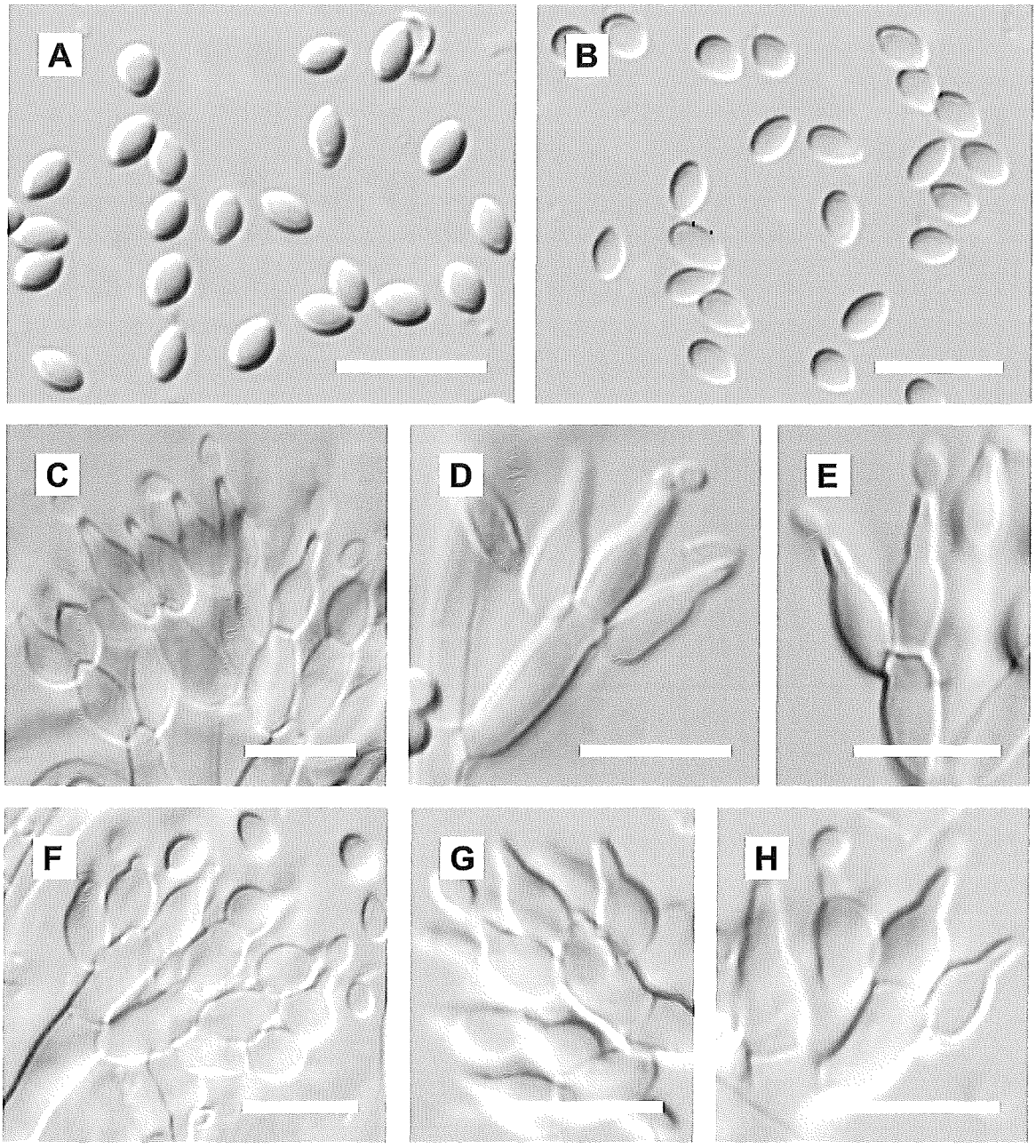

Figure 4.2. *Isaria farinosa:* **A** conidia, 060415.39; **C-E** phialides, 040610.1. *Isaria cf. farinosa:* **B** conidia, 060415.46; **F-H** phialides, 060511.10. Scale bars indicate 10 μm.

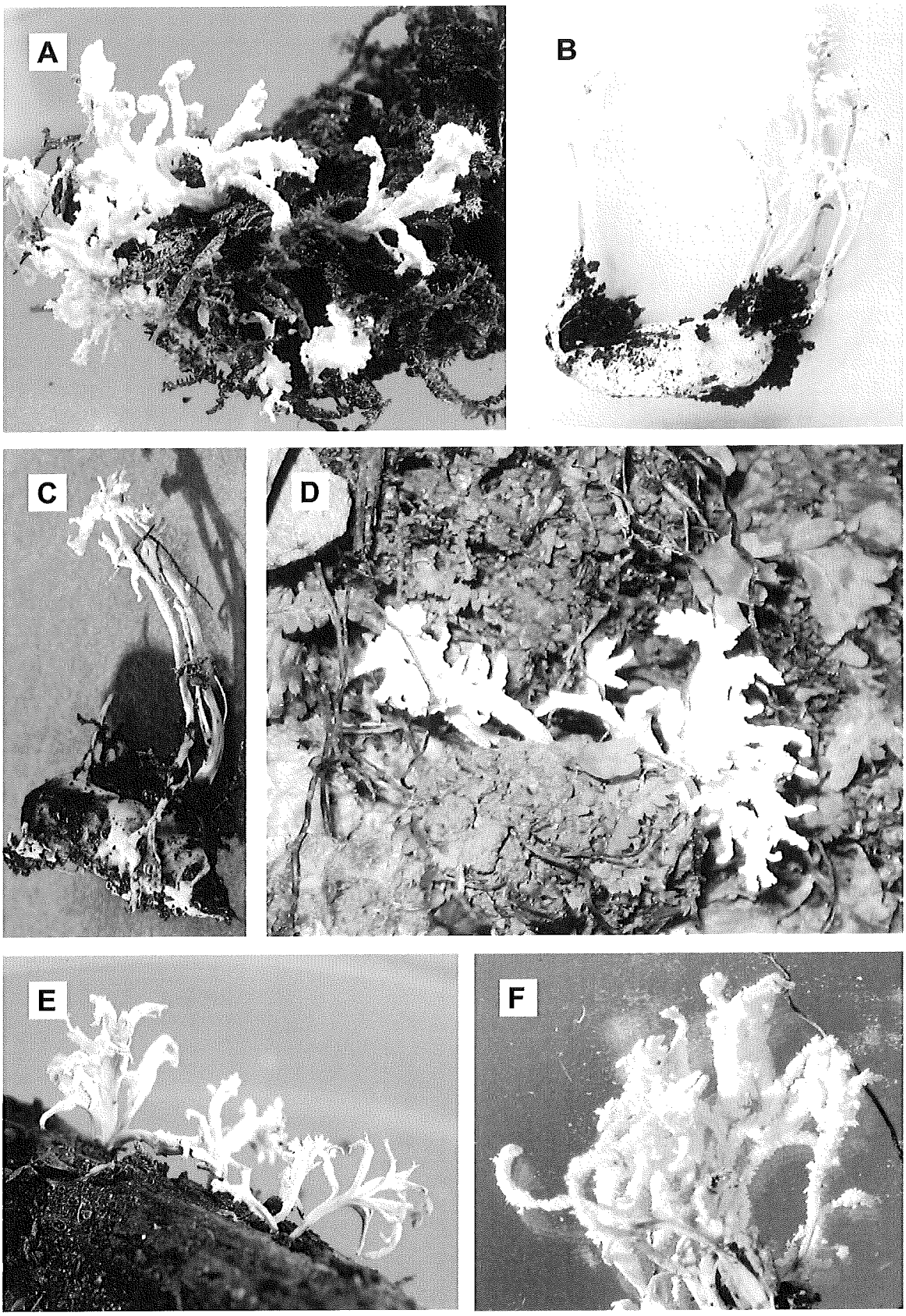

Figure 4.3. A-F: *Isaria tenuipes* on lepidopteran pupae. **A** 060415.45, **B** 030421.1, **C** 020417.1, **D** 060508.1, **E** 040610.2, **F** 020417.2.

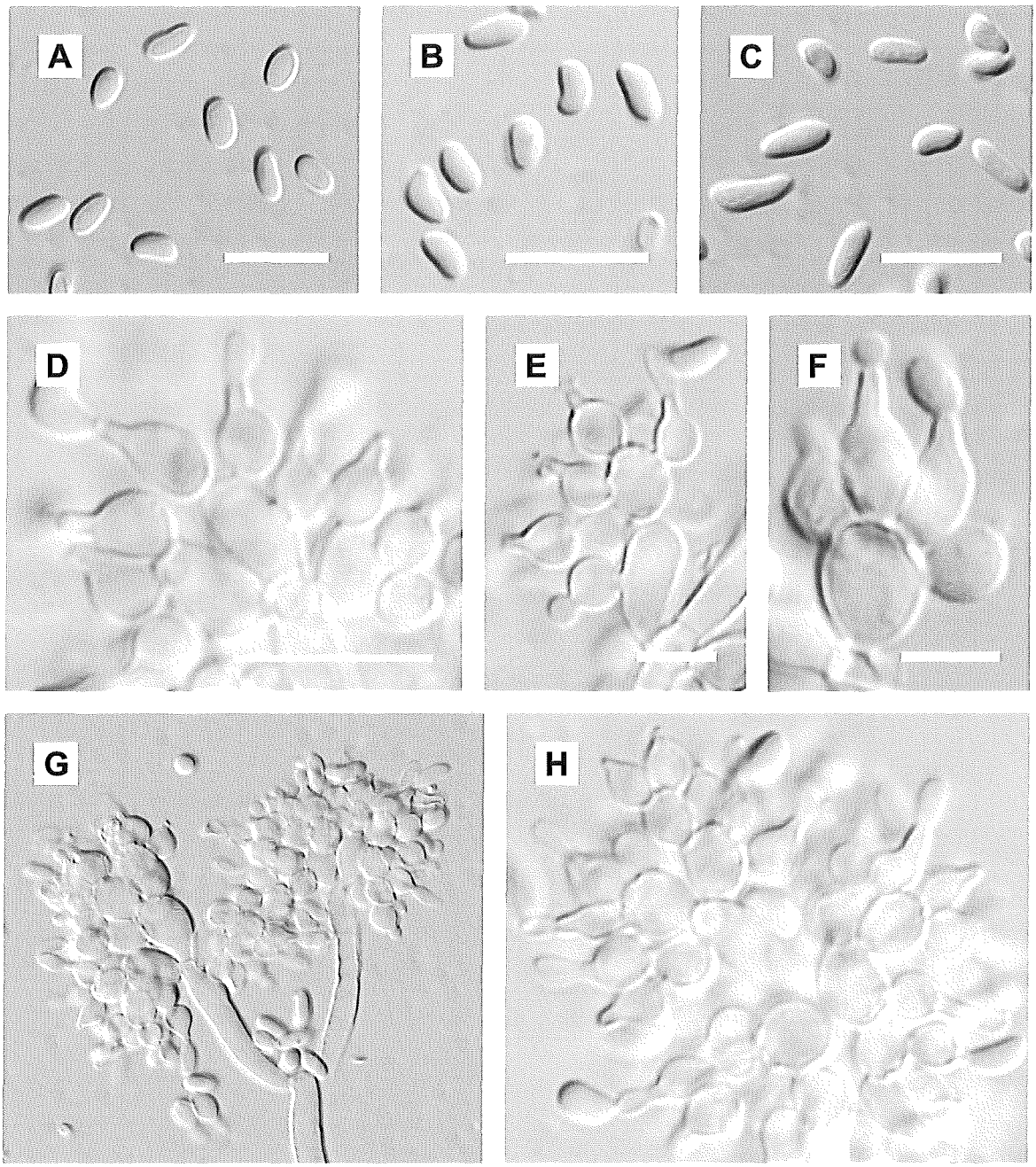

Figure 4.4. A-H: *Isaria tenuipes.* **A** conidia, 060508.1; **B** conidia, 020417.0; **C** conidia, 040610.3; **D-E** phialides, 030506.6; **F** phialides, 060415.45; **G** conidiophore and phialides, 060415.45; **(H)** conidiophore and phialides, 030506.6. Scale bars indicate 10μm, except E-F which are 5 μm.

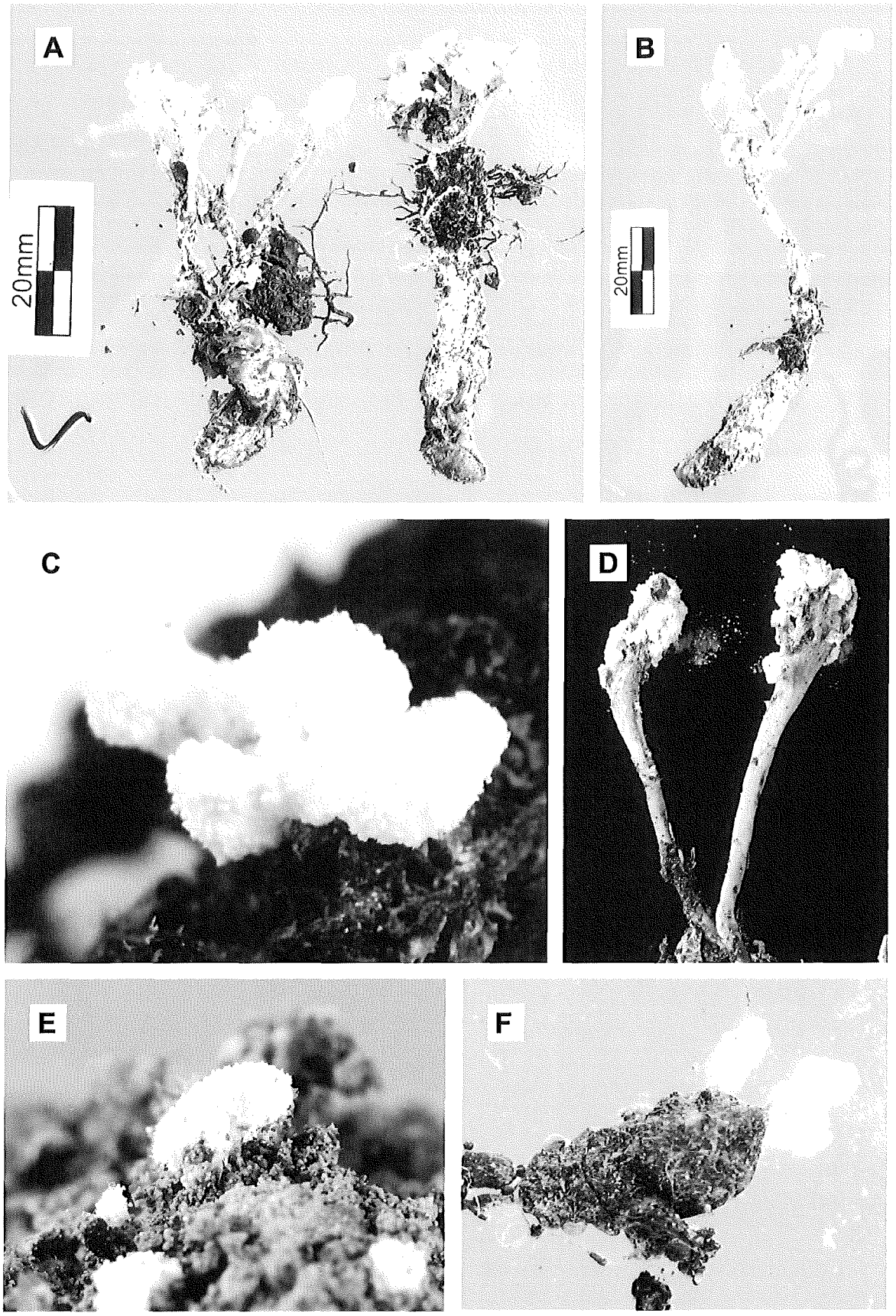

Figure 4.5. A-D *Isaria cicadae* on cicada nymphs: **A** 050302.3, 050302.4; **B** 050302.10; **C** 060508.7; **D** 050408.16. **E-F** *Isaria cf. cicadae*: **E** 050406.7, on lepidopteran pupa; **F** 050405.14, on Coleoptera.

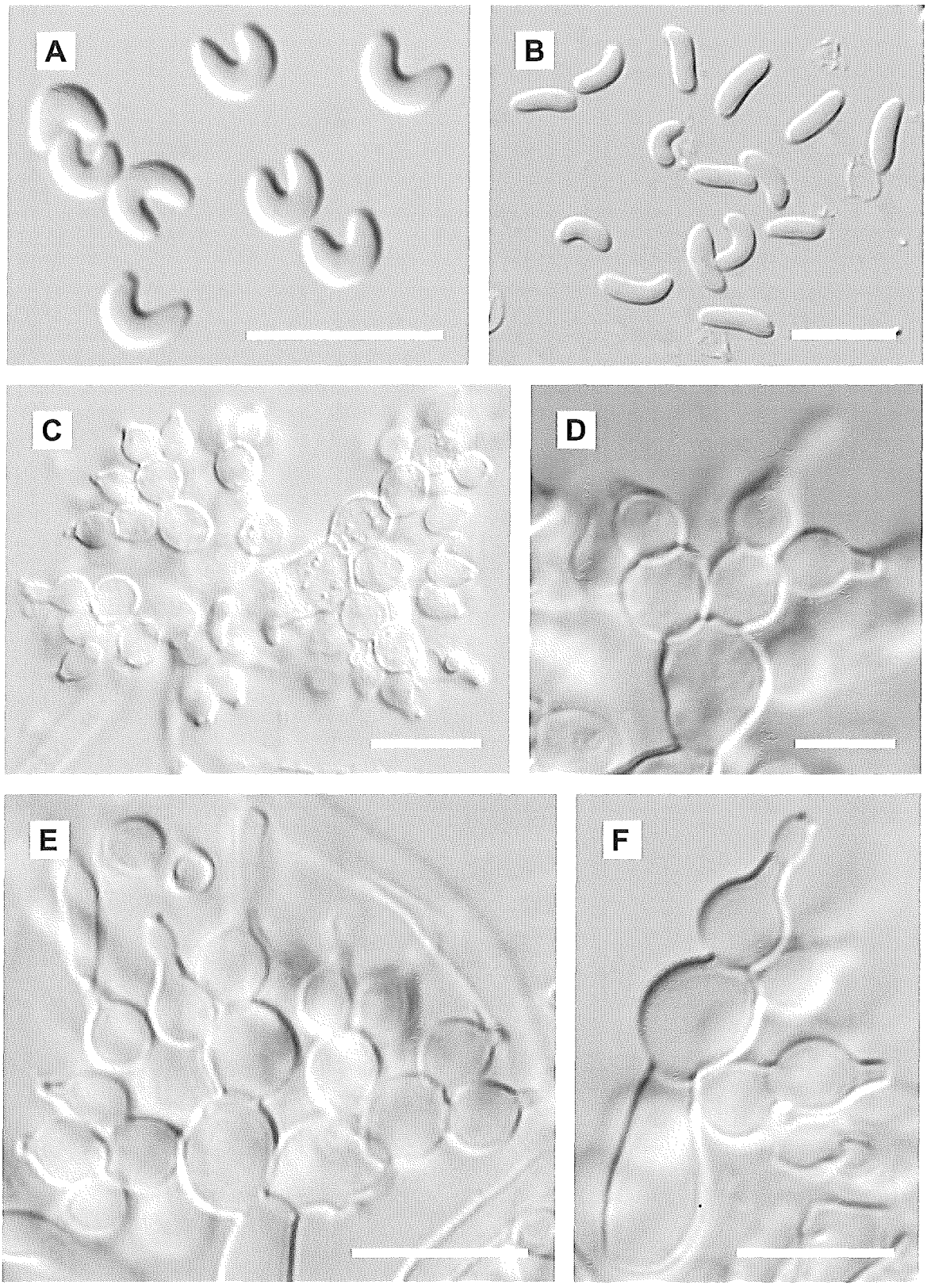

Figure 4.6. *Isaria cicadae*: **A** conidia, 050406.16; **C-D** conidiophores and phialides 050406.16. *Isaria cf. cicadae*: **B** conidia, 050405.14; **E-F** conidiophores and phialides, 050406.35. Scale bars indicate 10 μm.

4.3.3 Phylogenetic analyses

Sequences from the ITS1-5.8S-ITS2 region using primers ITS4 and ITS5 were approximately 534 nucleotides in length. The final ITS alignment consisted of 456 aligned positions, of which 63 were parsimony-informative sites. Maximum parsimony (MP) analysis of the ITS dataset generated 540 equally parsimonious trees with a length of 148 steps. Bayesian likelihood analysis was conducted using the GTR+I+G model. One of the most parsimonious trees from the maximum parsimony analysis of the ITS region is shown in Fig. 4.7 with MP bootstrap values (BS) and posterior probabilities (PP) from the Bayesian analysis indicating support for each clade.

The ITS phylogeny generally supported species identifications based on morphology, with New Zealand *Isaria* isolates clustering in four main clades. Isolates identified as *I. farinosa* grouped in a well supported basal clade (99% BS, 100% PP) with most overseas representatives of the taxon, but in a different clade from the type strain of *I. farinosa* from Denmark (CBS111113). Four isolates that were morphologically comparable to *I. farinosa* but characterised by larger spores (designated here as *I. cf. farinosa*) formed a distinct clade (80% BS, 100% PP) that grouped strongly (95% BS, 100% PP) with CBS111113. New Zealand isolates of *I. cicadae*-like fungi clustered with strains of *I. cicadae* from China and Japan, although MP analysis showed only limited bootstrap support (67% BS) for this clade and it was not resolved in the Bayesian analysis. MP and Bayesian analyses indicated a distinction between New Zealand strains of *I. cicadae* from cicadas and larger-spored strains (designated *I. cf. cicadae)* from non-cicada hosts, although again this was not strongly supported by bootstrap values (64%) or posterior probabilities (87%). New Zealand isolates of *I. tenuipes* grouped in a well supported clade (90% BS, 100% PP) with overseas strains of the species.

Partial sequences from the β-tubulin and EF1-α gene regions were analysed to further confirm the phylogenetic groupings indicated from ITS analysis. Sequences from the β-tubulin region using primers Bt2a and Bt2b were approximately 330 nucleotides in length. The final alignment had 299 aligned positions with 65 parsimony-informative sites. Maximum parsimony analysis of the ITS dataset yielded 106 equally parsimonious trees with a length of 142 steps. Bayesian likelihood analysis was conducted using the GTR+I model (general time reversible model with a proportion of invariable sites. One of the most parsimonious trees from maximum parsimony analysis of the partial β-tubulin region is shown in Figure 4.8A with MP bootstrap values (BS) and posterior probabilities (PP) from the Bayesian analysis indicating support for each clade.

Partial EF1-α sequences obtained using the primers 1777F and 2218R consisted of 529 nucleotides. The final alignment had 493 positions, including 108 parsimony-informative sites. Maximum parsimony (MP) analysis of the EF1-α dataset generated 107 equally parsimonious trees with a length of 180 steps. Bayesian likelihood analysis was conducted using the GTR+G model (general time reversible model with a gamma distribution). One of the most parsimonious trees from maximum parsimony analysis of the EF1-α region is shown in Fig. 4.8B with MP bootstrap values (BS) and posterior probabilities (PP) from the Bayesian analysis indicating support for each clade.

Analysis of partial EF1-α and β-tubulin regions also supported the distinction between the two groups of *I. farinosa*-like strains. In the β-tubulin phylogeny the larger spored isolates again formed a separate group (96% BS, 100% PP) in a clade with CBS 111113 (99% BS, 100% PP). The more typical *I. farinosa* isolates were clearly separated in a different clade (80% BS, 100% PP) with other overseas representatives. Although no sequence of CBS111113 was available for comparison in the EF1-α analysis two distinct groups were again supported with the larger-spored isolates forming a separate clade (99% BS, 100% PP) from a similarly well-supported (99% BS, 100% PP) group of NZ and overseas *I. farinosa*.

No EF1-α or β-tubulin sequences from overseas *I. cicadae* were available for phylogenetic comparison with NZ strains. Analysis of the partial β-tubulin region did not show any genetic variation among *I. cicadae*-like isolates from different hosts. However, the EF1-α phylogeny indicated (69% BS) that *I. cicadae* isolates from cicadas formed a distinct subgroup from the larger spored examples associated with Coleopteran and Lepidopteran hosts. However, as in the ITS analysis this distinction showed only limited bootstrap support (69% BS) in the MP analysis and was not resolved by Bayesian inference.

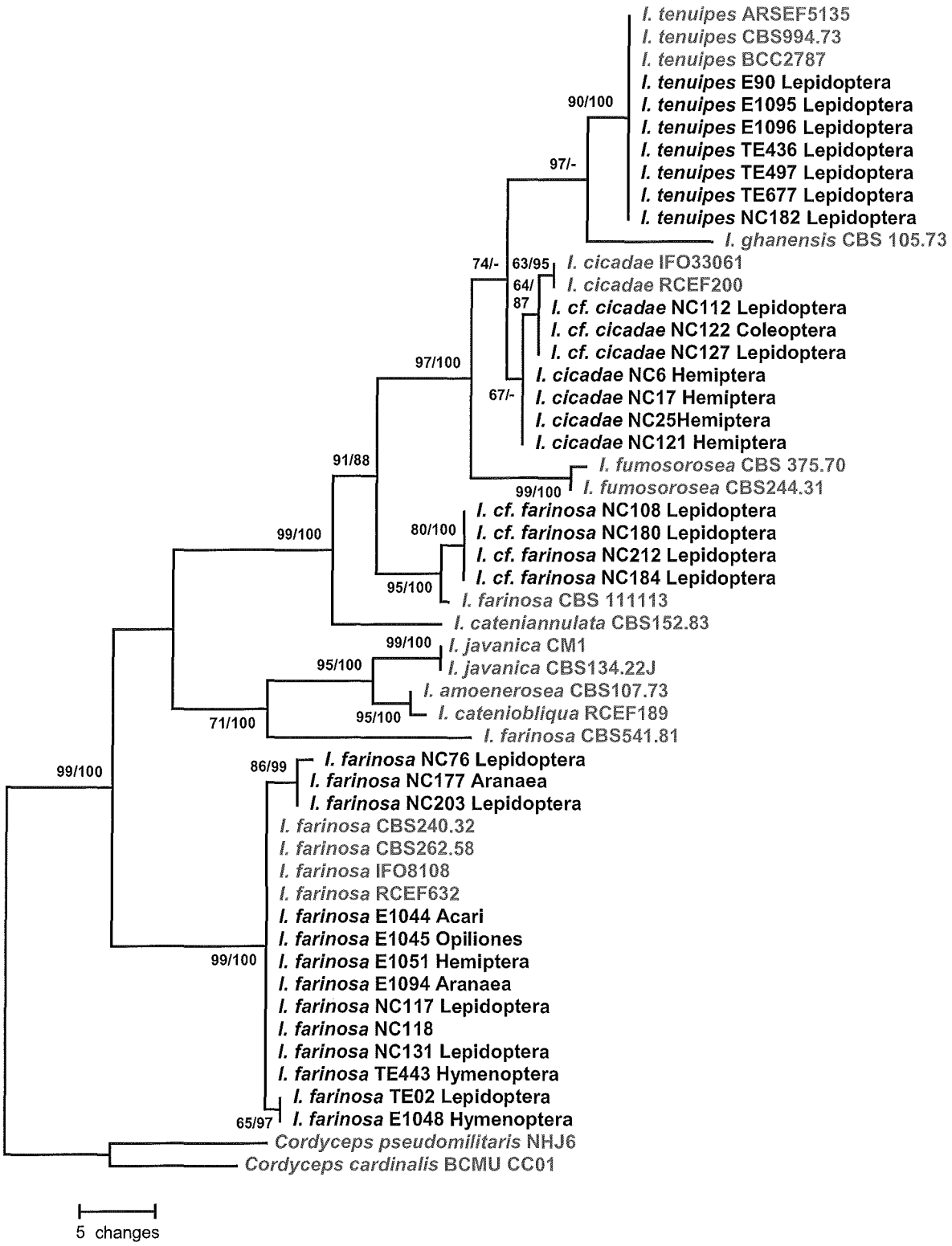

Figure 4.7 One of the most parsimonious trees showing relationships of New Zealand *Isaria* species with overseas strains (in red). The numbers at each branch represent bootstrap values based on 1000 replicates and posterior probabilities from Bayesian analysis, respectively. Only values over 50% are shown. Phylogenetic distance is indicated by the scale bar at the base of the figure.

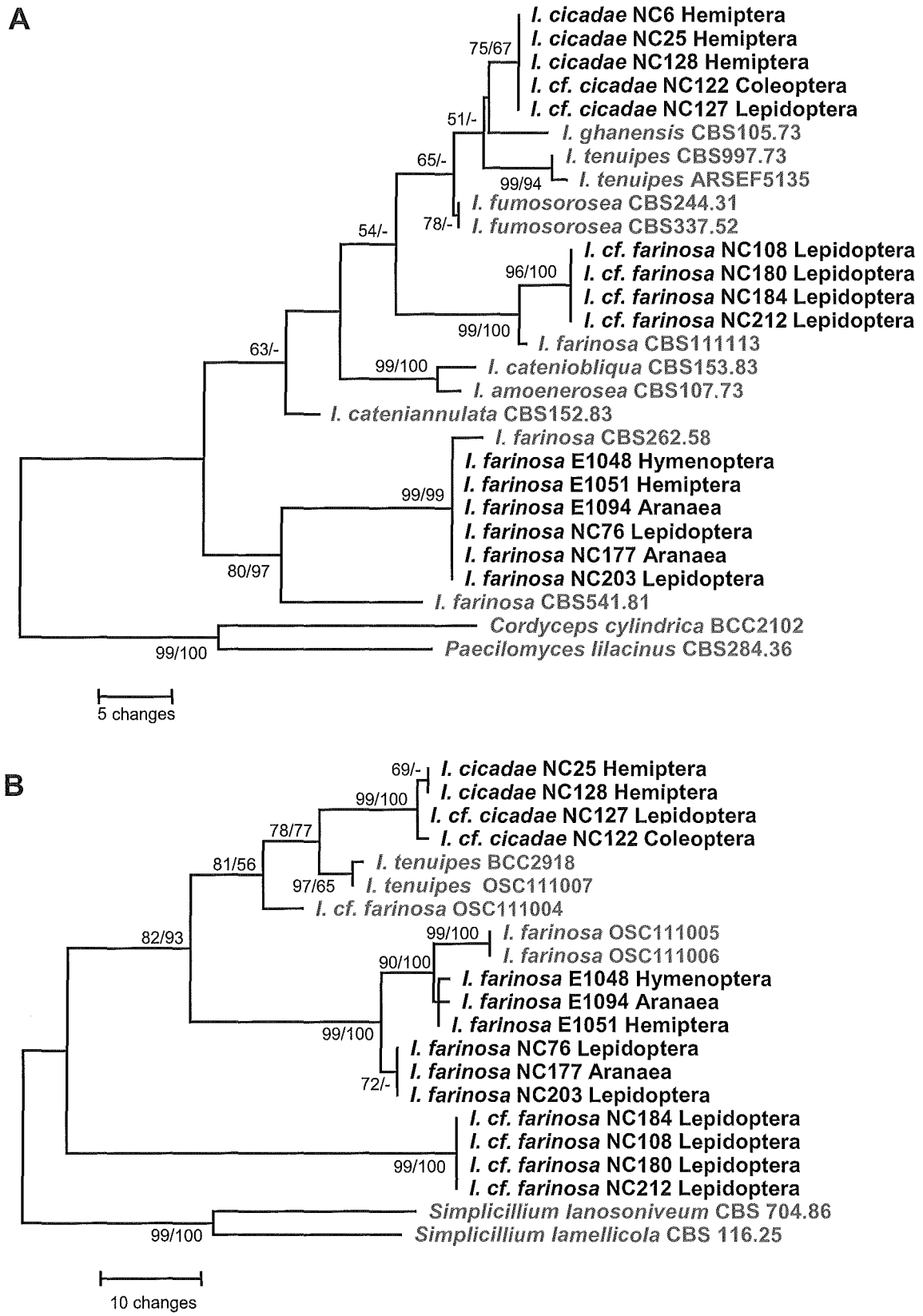

Figure 4.8 Relationships of New Zealand *Isaria* species with overseas strains (in red) based on analysis of (A) partial β-tubulin and (B) partial EF1-α sequences. Each tree is one of the shortest trees from maximum parsimony analysis with numbers at each branch denoting bootstrap values based on 1000 replicates and posterior probabilities from Bayesian analysis, respectively. Only values over 50% are shown. Phylogenetic distance is indicated by the scale bar at the base of each figure.

4.4 Discussion

Isaria species were found to be common in New Zealand native forests and were collected from sites in all of the five main regions in this study (Tables 4.3, 4.5, 4.6). Individual species varied in their host preferences and included generalist and host-specific pathogens. Insects were the most frequent hosts, primarily immature stages of Lepidoptera (42% of collections) and Hemiptera (36%). Several collections were also made from non-insect arthropods in Acari, Aranaea and Opiliones. (Tables 4.4, 4.6). *Isaria farinosa* and *Isaria cicadae* appeared to be the main *Isaria* species in native forest.

Isaria farinosa was the most commonly collected *Isaria* species in this study. While the species was most frequently found in association with lepidopteran larva and pupae it was also found to infect adult Lepidoptera and several wasp species including the introduced *Vespula vulgaris* (Hymenoptera: Vespidae). Mites (Acari), spiders (Araneae) and a harvestman (Opilionidae) were also found as hosts. The wide host range of *I. farinosa* among arthropods may reflect a role as an opportunistic pathogen capable of saprotrophic survival outside of the host. The species is often found in forest soils and litter (Domsch *et al.* 1980; Harney & Widden 1991b; Samson 1974; Sosnowska *et al.* 2004; Vanninen 1996) and has been shown to actively decompose plant litter (Harney & Widden 1991a).

Isaria farinosa often formed simple cylindrical to clavate synnemata on the host, generally with a yellowish or orange stipe and reaching lengths of up to 10 mm (Fig. 4.1A, D, E, G). However, in many cases synnemata were poorly differentiated (Fig. 4.1B) or not present (Fig. 4.1C, F). Synnemata formation in *I. farinosa* appears to be a response to environmental conditions rather than a genotypic trait associated with particular strains. Chew *et al.* (1998) found no correlation between genetic groups of *I. farinosa* and presence or absence of synnemata. While Mains (1955) suggested that synnemata size was influenced by the size of the host, the specific microhabitat of the host seems to be a major factor in determining synnematal form. In native forests the synnemata were associated with hosts buried in soil or encased in leaves. Infected arthropods collected from more exposed positions (*e.g.* attached to trees or on the forest floor) generally lacked prominent synnemata. Samson & Evans (1977) noted that *I. fumosorosea* normally only produced synnemata on concealed hosts and that synnematal production in culture was influenced by specific light conditions. Similarly, the spider pathogen *Nomuraea atypicola* forms synnemata on burrow-dwelling trapdoor spiders but only produces mononematous conidiophores on hunting and aerial web-building hosts (Coyle *et al.* 1990). As the obvious function of synnemata is to elevate conidiophores to a position favourable for dispersal of

conidia (Evans 1982), their production has become an ecological requirement for species which infect hosts that are buried or otherwise hidden (Samson & Evans 1977).

Isaria farinosa was primarily distinguished from other members of the genus examined in this study by its ellipsoidal to fusiform conidia (Fig. 4.2A) measuring 2.0-2.9 × 1.1-1.9 μm on the host and 2.1-3.2 × 1.1-2.0 μm in culture. Phialides had ellipsoidal bases which tapered to a thin neck (Fig. 4.2C-E), and measured 3.8-9.8 × 1.9-3.5 μm. Although phialides and conidia were significantly longer on non-insect hosts, this was not demonstrated in measurements from cultures. This may suggest the differences are related to variation in the nutritional composition of the two groups of hosts. Morphological characteristics of New Zealand representatives matched closely with the description of *Paecilomyces farinosus* by Samson (1974) who recorded conidia as measuring 2.0- 3.0 × 1.0- 1-8 μm and phialides as 5.0-15.0 × 1.2-2.5 μm.

Isaria farinosa was first described as *Ramaria farinosa* by Holm from Denmark in 1780, although none of his original specimens were preserved (Hodge *et al.* 2005). In the recent establishment of *I. farinosa* as the type species for *Isaria*, Hodge *et al.* (2005) designated Holm's original illustration as the lectotype for the species. However, as the figure lacks microscopic detail and could easily also represent *I. tenuipes*, a specimen and corresponding culture of *I. farinosa* from Denmark were selected as an epitype. A sequence from the epitype culture was included in the ITS phylogeny of Luangsa-ard *et al.* (2005), but did not group closely with other included representatives of the species, demonstrating that *I. farinosa* is not monophyletic within *Isaria*.

In the ITS phylogeny presented in this study, New Zealand isolates identified as *I. farinosa* grouped with examples of the species from various countries, forming a well-supported clade at a basal position in the tree that was distinct from the epitype strain (CBS 111113). Analysis of partial β-tubulin sequences also clustered New Zealand isolates with overseas strains of *I. farinosa* in a different clade from the type. New Zealand isolates again grouped with overseas strains of *I. farinosa* in the EF1-α analysis, although a sequence from the type culture was not available for comparison. The majority of New Zealand ITS sequences were identical to most sequences from overseas strains, although others diverged by several nucleotides. Three isolates (NC76, NC177, NC203) formed a well supported subclade in the ITS and EF1-α phylogenies but this did not correlate with any differences in conidial size or host affiliation. All β-tubulin sequences from examined isolates of NZ *I. farinosa* were identical.

Four isolates from lepidopteran pupae (NC108, NC180, NC184, NC212) were morphologically similar to *I. farinosa* with ellipsoidal to fusiform conidia (Fig. 4.2B), but conidia were found to

be larger, measuring 2.5-3.8 × 1.5-2.5 µm on the host material and 2.6-4.0 × 1.8-2.7 µm in culture. The arrangement of conidiogenous structures was similar to that observed in specimens identified as *I. farinosa*, although phialides typically had a wider base, ranging from 2.3-4.0 µm (Fig 4.2F-H). All of the collected material was characterised by the production of simple white synnemata on the host (Fig. 4.1H). Molecular analyses placed these apart from other isolates in a distinct clade which was well-supported in the ITS, EF1-α, and β-tubulin phylogenies. A close affiliation with the epitype strain of I. *farinosa* (CBS111113) was shown from analysis of the ITS and β-tubulin regions. Both morphological and molecular data clearly indicates that these isolates should be placed in a separate species from the more commonly encountered *I. farinosa* with smaller spores. However, the taxonomic status of "*I. farinosa*" seems uncertain and molecular data from this study and from Luangsa-ard *et al.* (2005) indicates that the strain selected as the type may not be an ideal representative of the species. Currently, the taxon appears to be a species complex that requires further phylogenetic revision.

Isaria tenuipes was collected and isolated from lepidopteran pupae buried under moss or in leaf litter. The species appeared to be less common than *I. farinosa* or *I. cicadae*, which may explain the scarcity of previous New Zealand records. Typically, the species produced characteristic synnemata which were highly branched with bright yellow stipes, extending up to about 20 mm from the host (Fig. 4.3). Synnemata of *I. tenuipes* have often been described as resembling *I. farinosa* (Bissett 1979a; Mains 1955; Samson 1974). However, as also noted by Petch (1937), the examined specimens had conidial heads that were composed of distinct terminal branches and were less compact than in *I. farinosa*, giving a characteristic plumose appearance. This was especially pronounced in older specimens that had lost most of their conidia.

Conidia of *I. tenuipes* were ellipsoidal to cylindrical, often slightly curved or allantoid (Fig. 4.4A-C). Conidial dimensions showed some variation on the host, with conidia of most specimens ranging from 3.2-5.4 × 1.2-2.4 µm, while several collections had conidia up to 6.9 µm long. In culture, conidia measured 3.9-9.1 × 1.4-3.5 µm. Samson (1974) recorded *P. tenuipes* strains as having two distinct types of conidia: either one-celled and measuring 3-7.5 x 2.0-2.5 µm or 6-12 µm long with one or two cells. No two-celled conidia were seen in the New Zealand isolates. Phialides generally measured 3.6-6.6 × 2.7-5.3 µm and had a globose-subglobose base, tapering abruptly or gradually to a short, thin neck (Fig. 4.4D-F). Metulae were typically globose-subglobose and borne on swollen, densely branched conidiophores (Fig. 4.4G-H). Morphology closely matched the description of the species in Samson (1974). ITS sequences of the New Zealand isolates showed no variation and were identical to sequences from overseas strains, including a culture derived from the type locality in North America (ARSEF5135).

All *Isaria* species on cicada nymphs collected in native forest were identified as *Isaria cicadae*. The species was often observed in large numbers, and at one site over 50 individual specimens were counted in an area covering about 25 square metres. In most cases *I. cicadae* could be easily identified before microscopic examination due to its specific host and characteristic stout, branched synnemata which reached up to 70 mm long with conidia aggregated in compact, cauliflower-like heads (Fig. 4.5A-D). Conidia from host material were cylindrical and usually highly curved (Fig. 4.6A), measuring from 3.2-6.5 × 1.1-2.7 µm, which corresponds closely with *I. cicadae* according to Samson (1974). Conidia in culture were extremely variable; some were similar in size and shape to those from the host while others were cylindrical and straight or irregularly curved, sometimes with a tunicate (hook-shaped) or sigmoid appearance, and measuring up to 12.3 µm long. As shown by Kobayasi (1939, 1941), Kobayasi & Shimizu (1963), and Samson (1974), conidiogenous structures (Fig. 4.6C-D) strongly resembled those of *I. tenuipes*, with both species producing phialides with a globose-subglobose base and a short, thin neck from globose-subglobose metulae and densely branched, highly swollen conidiophores.

Three isolates (NC122, NC112, NC127) from hosts other than cicadas (lepidopteran pupae and a coleopteran species) produced cylindrical, curved conidia (Fig. 4.6B) measuring 5.7-9.0 × 1.6-2.5 µm on the host and 6.2-13.7 × 1.4 -3.0 µm in culture. Phialides were similar to those observed on cicada specimens (Fig. 4.6E-F). While these also tend to fit with Samson's description of *I. cicadae* they were clearly different from the New Zealand specimens described from cicada nymphs. Although one specimen had similar synnemata to those produced on cicadas, the other collections had greatly reduced synnemata that extended only a few millimeters from the host (Fig. 4.5E-F). Analysis of ITS and EF1-α sequences also supported the distinction between the two groups of New Zealand *I. cicadae*-like isolates, although differences were limited to two nucleotides in each region.

Isaria cicadae was first described by Miquel in 1838 for a fungus infecting buried cicada nymphs in Brazil (Petch 1933). The species received little attention until it was considered by Petch to be an earlier synonym of *Isaria sinclairii* which had been originally described from New Zealand material as *Cordyceps sinclairii* by Berkeley (1855). Although the original description of *C. sinclairii* did not include a figure the species was later illustrated in Berkeley (1857) and also (in more detail) by Gray (1858). Berkeley (1855) recorded the fungus as occurring on an "orthopterous insect"; however Gray (1858) was more definite in stating that the host was a cicada. *Torrubia caespitosa*, described from cicadas by Tulasne in 1865, was apparently based on the same set of specimens examined by Berkeley (Cooke 1892; Lloyd

1915). A fungus illustrated by Taylor (1855) from New Zealand and (invalidly) named as *Sphaeria basili* also appears to be the same species. The host was given by Taylor as a "locust", a term often used in the nineteenth century to describe cicadas (Kritsky 2001).

Massee (1895) and Cunningham (1921) both noted that *C. sinclairii* had never been shown to produce perithecia and the species was accordingly recombined in *Isaria* by Lloyd (1923). The description by Berkeley (1855) of *Cordyceps sinclairii* gave little detail of microscopic characters, although spores were given as about 7 µm long and oblong-shaped. Petch (1924) provided the first detailed description of *Isaria sinclairii* from specimens collected in Ceylon. Conidia were described as oblong-oval measuring 8.0-10.0 × 2.0-3.0 µm. Petch (1933) later examined specimens of *I. sinclairii* from New Zealand and Mexico, and deciding that these were the same as the species earlier described by Miquel, synonymised *I. sinclairii* and several other species with *I. cicadae*. Kobayasi (1939) however, disagreed with Petch and preferred to retain the name *I. sinclairii*, stating that *I. cicadae* was "obscurely known with inadequate description based on the sterile and dried specimen". Conidia of *I. sinclairii* were recorded from Japanese material by Kobayasi (1939, 1941) and Kobayasi & Shimizu (1963) as ovoid, elongate-ellipsoidal, or fusiform; frequently curved; and measuring 5.0-9.0 × 2.0-3.0 µm.

In transferring entomopathogenic *Isaria* species to *Paecilomyces*, Samson (1974) followed Petch (1933) and made the combination *Paecilomyces cicadae*, giving spore dimensions as 3.5-8.0 × 1.5-3.5 µm. Samson examined specimens from various countries including a New Zealand collection of *Cordyceps sinclairii* from Berkeley's herbarium. Samson (1974 fig. 20) illustrated three sets of conidia for *P. cicadae*: two groups equate to the large cylindrical-ellipsoidal conidia as described for *I. sinclairii* from Ceylon (Petch 1924) and Japan (Kobayasi 1939), while the third set are identical to the smaller, highly curved conidia observed for New Zealand *I. cicadae* in the present study. Although Samson does not reference these conidial types to any particular specimen it seems likely that the smaller conidia illustrated are from the New Zealand specimens he examined.

It is suggested that New Zealand strains identified as *Isaria cicadae*, with conidia measuring 3.2-6.5 × 1.1-2.0 µm may represent a separate species to similar fungi with larger conidia recorded from cicadas in other parts of the world. New Zealand isolates with large conidia from hosts other than cicadas appear to be an allied species that may be more closely related to overseas *I. cicadae*. The morphological distinction between the two groups is also supported to a limited extent by phylogenetic data, although a lack of available sequences prevented a detailed molecular comparison with strains from other countries. Further examination of New Zealand

and overseas isolates using both morphology and multiple, high-resolution molecular markers is clearly necessary to clarify the taxonomy of this species.

CONCLUSIONS

Species of *Beauveria* and *Isaria* were found to be frequently occurring in native forests and must function as important natural regulators of arthropod populations in these habitats. The two recognized phylogenetic species (clades A and C) within *Beauveria bassiana* were both found to be present in native forests. Clade C has not been identified from disturbed habitats in this country and it is suggested that the group is restricted to natural forests in New Zealand. *Beauveria malawiensis*, previously only known from coleopteran hosts, was found to have a broad host range in native forests, infecting representatives of the insect orders Hemiptera, Hymenoptera, Orthoptera and Phasmatodea. It is suggested that many previous records of *Beauveria brongniartii* in this country may have been *B. malawiensis*. Insect bioassays generally indicated that individual strains of *B. malawiensis* and *B. bassiana* are not highly host-specific and demonstrated the potential of the both species for the biological control of *Vespula* wasps. *Beauveria caledonica*, previously known only from pine forests in New Zealand, was also found to be established in native forests. The species was also recorded for the first time from non-coleopteran hosts. *Isaria farinosa* and *I. tenuipes*, although poorly recorded in New Zealand, were both present in native forests. Molecular data supported previous findings that *I. farinosa* is not monophyletic and may consist of an assemblage of morphologically similar species. Two distinct groups of *I. cicadae*-like fungi were identified in native forests. It is suggested that species from cicada nymphs in New Zealand previously classified as *I. cicadae* may represent a separate, possibly endemic species.

Molecular analyses presented in this study have indicated several unique lineages in New Zealand representatives of *Beauveria* and *Isaria* species. Future studies using additional molecular markers are necessary to provide further insight into the diversity and phylogeographic origins of these species in New Zealand.

REFERENCES

Andersen, S., Hojrup, P. & Roepstorff, P. (1995). Insect cuticular proteins. *Insect Biochemistry and Molecular Biology* **25**, 153-176.

Anon (1892). Entomological. White grub culture. *Otago Witness* 24/11/1892, p5.

Anon (1893). Entomological. *Sporotrichum globuliferum. Otago Witness* 4/4/1893, p5.

Anon (2001-2009) [Herbarium PDD]. *NZFUNGI Database of New Zealand Fungi.* http://nzfungi.landcareresearch.co.nz/html/mycology.asp.

Aquino de Muro, M.A., Elliott, S., Moore, D., Parker, B.L., Skinner, M., Reid, W. & El Bouhssini, M. (2005). Molecular characterisation of *Beauveria bassiana* isolates obtained from overwintering sites of Sunn Pests (*Eurygaster* and *Aelia* species). *Mycological Research* **109**, 294-306.

Aquino de Muro, M.A., Mehta, S. & Moore, D. (2003). The use of amplified fragment length polymorphism for molecular analysis of *Beauveria bassiana* isolates from Kenya and other countries, and their correlation with host and geographical origin. *FEMS Microbiology Letters* **229**, 249-257.

Baker, G. (1985). Parasites of the millipede *Ommatoiulus moreletti* (Lucas) (Diplopoda: Lulidae) in Portugal, and their potential as biological control agents in Australia. *Australian Journal of Zoology* **33**, 23-32.

Baker, R. (1981). Natural mortality of currant clearwing, *Synanthedon salmachus* (Lepidoptera: Sesiidae), in New Zealand. *New Zealand Journal of Zoology* **8**, 529-530.

Barker, G. & Addison, P. (1989). Sampling Argentine stem weevil, *Listronotus bonariensis* (Kuschel), populations in pasture: the soil-dwelling stages. *New Zealand Journal of Agricultural Research* **32**, 95-103

Barker, C.W. & Barker, G.M. (1998). Generalist entomopathogens as biological indicators of deforestation and agricultural land use impacts on Waikato soils. *New Zealand Journal of Ecology* **22**, 189-196.

Barker, G.M., Goh, H.H., Lyons, S.N. & Addison, P.J. (1991). Comparative pathogenicity to Argentine stem weevil of *Beauveria bassiana* from various hosts. *Proceedings of the NZ Weed and Pest Control Conference* **44**, 214-215.

Barlow, N. & Goldson, S. (2002). Alien invertebrates in New Zealand. In *Biological invasions - Environmental impacts* (ed. D. Pimentel) pp. 195-217. CRC Press: Boca Raton.

Baverstock, J., Clark, S.J. & Pell, J.K. (2008). Effect of seasonal abiotic conditions and field margin habitat on the activity of *Pandora neoaphidis* inoculum on soil. *Journal of Invertebrate Pathology* **97**, 282-290.

Beggs, J.R. (2001). The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. *Biological Conservation* **99**, 17-28.

Beggs, J.R., Rees, J.S. & Harris, R.J. (2002). No evidence for establishment of the wasp parasitoid, *Sphecophaga vesparum burra* (Cresson) (Hymenoptera: Ichneumonidae) at two sites in New Zealand. *New Zealand Journal of Zoology* **29**, 205-211.

Beggs, J.R., Rees, J.S., Toft, R.J., Dennis, T.E. & Barlow, N.D. (2008). Evaluating the impact of a biological control parasitoid on invasive *Vespula* wasps in a natural forest ecosystem. *Biological Control* **44**, 399-407.

Beggs, J.R., Toft, R.J., Malham, J., Rees, J.S., Tilley, J., Moller, H. & Alspach, P.A. (1998). The difficulty of reducing introduced wasp (*Vespula vulgaris*) populations for conservation gains. *New Zealand Journal of Ecology* **22,** 55-63.

Beggs, J.R. & Wilson, P. (1991). The kaka, *Nestor meridionalis*, a New Zealand parrot endangered by introduced wasps and mammals. *Biological Conservation* **56**, 23-38.

Berkeley, M. (1855). Nat. Ord. CII. Fungi. In *The Botany of the Antarctic Voyage II. Flora Novae-Zelandiae. Part II. Flowerless Plants* (eds. J. Hooker) pp. 172-210. Lovell Reeve: London.

Berkeley, M. (1857). *Introduction to cryptogamic botany.* H. Bailliere: London.

Bidochka, M.J, Kamp, A.M., Lavender, T.M., Dekoning, J., & De Croos, J.N. (2001). Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? *Applied and Environmental Microbiology* **67**, 1335-1342.

Bidochka, M.J., McDonald, M.A., St Leger, R.J. & Roberts, D.W. (1994). Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA(RAPD). *Current Genetics* **25**, 107–113.

Bidochka, M.J., Menzies, F.V. & Kamp, A.M. (2002). Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. *Archives of Microbiology* **178**, 531-537.

Bidochka M.J. & Small, C. (2005). Phylogeography of *Metarhizium*, an insect pathogenic fungus. In *Insect-fungal associations: ecology and evolution* (eds. F.E. Vega & M. Blackwell) pp. 28-50. Oxford University Press: Oxford.

Bissett, J. (1979a). *Paecilomyces tenuipes. Fungi Canadenses* **158**, 1-2.

Bissett, J. (1979b). *Paecilomyces breviramosus. Fungi Canadenses* **159**, 1-2.

Bissett, J. & Widden, P. (1988). A new species of *Beauveria* isolated from Scottish moorland soil. *Canadian Journal of Botany* **66**, 361-362.

Booth, C. (1961). Studies of Pyrenomycetes: VI. *Thielavia*, with notes on some allied genera. *Mycological Papers* **83**, 1-15.

Boucias, D.G. & Pendland, J.C. (1984). Nutritional requirements for conidial germination of several host range pathotypes of the entomopathogenic fungus, *Nomuraea rileyi*. *Journal of Invertebrate Pathology* **43**, 288-292.

Boucias, D.G. & Pendland, J.C. (1991). Attachment of mycopathogens to cuticle: the initial event of mycoses in arthropod hosts. In *The fungal spore and disease initiation in plants and animals* (eds. G. Cole & H. Hoch) pp. 101-128. Plenum Press: New York.

Boucias, D.G., Pendland, J.C. & Latge, J.P. (1988). Nonspecific Factors Involved in Attachment of Entomopathogenic Deuteromycetes to Host Insect Cuticle. *Applied and Environmental Microbiology* **54**, 1795-1805.

Bridge, P., Abraham, Y., Cornish, M., Prior, C. & Moore, D. (1990). The chemotaxonomy of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates from the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). *Mycopathologia* **111**, 85-90.

Bridge, P.D., Spooner, B. & Roberts, P. (2005). The impact of molecular data in fungal systematics. *Advances in Botanical Research* **42**, 33-67.

Brito, M. (1994). The millipede of Santo Antao, *Spinotarsus caboverdus*: survey for pathogenic microorganisms, bioassay tests against *S. caboverdus* and *Melanoplus sanguinipes*. Unpublished M.Sc. thesis, Montana State University, Boseman.

Brown, A. & Smith, G. (1957). The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. *Transactions of the British Mycological Society* **40**, 17-89.

Brown, J. & Hovmoller, M. (2002). Aerial dispersal of pathogens in the global and continental scales and its impact on plant disease. *Science* **297**, 537-541.

Brownbridge, M., Nelson, T.L., Hackell, D.L., Eden, T.M., Wilson, D.J, Willoughby, B.E. & Glare, T.R. (2006). Field application of biopolymer-coated *Beauveria bassiana* F418 for clover root weevil (*Sitona lepidus*) control in Waikato and Manawatu. *New Zealand Plant Protection* **59**, *304-31.*

Bruns, T. (2001). ITS reality. *Inoculum* **52**, 2-3.

Bruns, T. & Shefferson, R. (2004). Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. *Canadian Journal of Botany* **82**, 1122-1132.

Bruns, T., White, T. & Taylor, J. (1991). Fungal molecular systematics. *Annual Review of Ecology and Systematics* **22**, 525-564.

Buchanan, P.K., Beever, R.E., Galloway, D.J., Glare, T.R., Johnston, P.R., McKenzie, E.H.C., di Menna, M.E., Pennycook, S.R., Ridley, G.S., Smith, J.M.B. & Stephenson, S.L. (2004). The fungi of New Zealand : an introduction. In *Introduction to Fungi of New Zealand* (ed. E. McKenzie) pp. 1-48. Fungal Diversity Press: Hong Kong.

Butt, T.M. (2002). Use of entomogenous fungi for the control of insect pests. In *The Mycota XI. Agricultural applications* (ed. F. Kempen) pp. 111-134. Springer-Verlag: Berlin.

Bye, N. & Charnley, A. (2008). Regulation of cuticle-degrading subtilisin proteases from the entomopathogenic fungi, *Lecanicillium* spp.: implications for host specificity. *Archives of Microbiology* **189**, 81-92.

Cameron, P.J. & Walker, G.P. (1989). *Acyrthosiphon kondoi* Shinji, blue-green lucerne aphid and *Acyrthosiphon pisum* (Harris), pea aphid (Homoptera: Aphididae). In *A review of biological control of invertebrate pests and weeds in New Zealand 1874-1987* (eds.

P.J. Cameron, R.L. Hill, J. Bain & W.P. Thomas) pp. 3-8. CAB International, Wallingford.

Carruthers, R., Sawyer, A. & Hural, K. (1991). Use of fungal pathogens for biological control of insect pests. In *Sustainable agriculture research and education in the field* (eds. B. Rice) pp. 336-372. National Academy Press: Washington.

Castellanos-Moguel, J., Gonzales-Barajas, M., Mier, T., del Rocio Reyes-Montes, M., Aranda, E. & Toriello, C. (2007). Virulence testing and extracellular subtilisin-like (Pr1) and trypsin-like (Pr2) activity during propagule production of *Paecilomyces fumosoroseus* isolates from whiteflies (Homoptera: Aleyrodidae). *Revista Iberoamericana de Micologia* **26**, 62-68.

Castrillo, L.A., Vandenberg, J.D. & Wraight, S.P. (2003). Strain-specific detection of introduced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers. *Journal of Invertebrate Pathology* **82**, 75-83.

Castrillo, L.A., Wiegmann, B.M. & Brooks, W.M. (1999). Genetic variation in *Beauveria bassiana* populations associated with the darkling beetle, *Alphitobius diaperinus*. *Journal of Invertebrate Pathology* **73**, 269-275.

Chandler, D., Davidson, G., Grant, W., Greaves, G. & Tatchel, G. (2008). Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory stability. *Trends in Food Science and Technology* **19**, 275-283.

Chandler, D., Davidson, G., Pell, J.K., Ball, B., Shaw, K. & Sunderland, K. (2000). Fungal biocontrol of Acari. *Biocontrol Science and Technology* **10**, 357-384.

Charnley, A. (1991). Microbial pathogens and insect pest control. *Letters in Applied Microbiology* **12**, 149-157.

Charnley, A. (2003). Fungal pathogens of insects: cuticle degrading enzymes and toxins. *Advances in Botanical Research* **40**, 241-321.

Charnley, A. & Collins, S. (2007). Entomopathogenic fungi and their role in pest control. In *The Mycota IV. Environmental and microbial relationships* (eds. C. Kubicek & I. Druzhinina) pp. 159-187. Springer-Verlag: Berlin.

Chaverri, P., Bischoff, J.F., Evans, H.C. & Hodge, K.T. (2005). *Regiocrella*, a new entomopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the Clavicipitaceae. *Mycologia* **97**, 1225-1237.

Chaverri, P., Liu, M. & Hodge, K.T. (2008). A monograph of the entomopathogenic genera *Hypocrella*, *Moelleriella*, and *Samuelsia* gen. nov. (Ascomycota, Hypocreales, Clavicipitaceae), and their aschersonia-like anamorphs in the Neotropics. *Stud Mycol* **60**, 1-66.

Chew, J.S.K., Strongman, D.B. & Mackay, R.M. (1998). Comparisons of twenty isolates of the entomopathogen *Paecilomyces farinosus* by analysis of RAPD markers. *Mycological Research* **102**, 1254-1258.

Cist, J. (1824). Notice of the *Melolontha* or may bug. *American Journal of Science and Arts* **8**, 269-271.

Clapperton, B., Lo, P.L., Moller, H., Sandlant, G.R. (1989). Variation in colour markings of German wasps *Vespula germanica* (F.) and common wasps *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) in New Zealand. *New Zealand Journal of Zoology* **16**, 303-313.

Clarkson, J.M. & Charnley, A. (1996). New insights into the mechanisms of fungal pathogenesis in insects. *Trends in Microbiology* **4**, 197-203.

Clements, F.E. & Shear, C.L. (1931). *The genera of fungi.* Hafner Press: New York.

Close, R. (1956). A muscardine fungus on a caterpillar of *Antheraea eucalypti* Scott (Lepidoptera, Saturniidae). *New Zealand Entomologist* **2**, 1-2.

Cokendolpher, J. (1993). Pathogens and parasites of Opiliones (Arthropoda: Arachnida). *The Journal of Arachnology* **21**, 120-146.

Cook, R.J., Bruckart, W.L., Coulson,J.R., Goettel, M.S., Humber, R.A., Lumsden, R.D., Maddox., J.V., McManus., M.L., Moore M., L., Meyer, S.F., Quimby, P.C., Stack J.P., Vaughn, J.L. (1996). Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. *Biological Control* **7**, 333-351.

Cooke, M.C. (1892). *Vegetable wasps and plant worms. A popular history of entomogenous fungi, or fungi parasitic upon insects.* Society for promoting Christian knowledge: London.

Coyle, F.A., Goloboff, P.A. & Samson, R.A. (1990). *Actinopus* trapdoor spiders (Araneae, Actinopodidae) killed by the fungus, *Nomuraea atypicola* (Deuteromycotina). *Acta Zoologica Fennica* **190**, 89-93.

Crosby, T., Dugdale, J. & Watt, J. (1976). Recording specimen localities in New Zealand: an abitrary system of areas and codes defined. *New Zealand Journal of Zoology* **3**, 69.

Crosby, T., Dugdale, J. & Watt, J. (1998). Area codes for recording specimen localities in the New Zealand subregion. *New Zealand Journal of Zoology* **25**, 175-183.

Crowe, A. (2002). *Which New Zealand insect?* Penguin Books: Auckland.

Cruz, L.P., Gaitan, A.L., Gongora, C.E., AF Cruz, L.P., Gaitan, A.L. & Gongora, C.E. (2006). Exploiting the genetic diversity of *Beauveria bassiana* for improving the biological control of the coffee berry borer through the use of strain mixtures. *Applied Microbiology and Biotechnology* **71**, 918-926.

Cunningham, G. (1921). The genus *Cordyceps* in New Zealand. *Transactions and Proceedings of the New Zealand Institute* **53**, 372-382.

Cunningham, G. (1922). A singular *Cordyceps* from Stephen Island, New Zealand. *Transactions of the British Mycological Society* **8**, 72-75.

Curran, J., Driver, F., Ballard, J. & Milner, R. (1994). Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. *Mycological Research* **98**, 547-552.

de Faria, M. & Wraight, S. (2007). Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* **43**, 237-256.

de Hoog, G. (1972). The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. *Studies in Mycology* **1**, 1-41.

de Hoog, G. (1978). Notes on some fungicolous Hyphomycetes and their relatives. *Persoonia* **10**, 33-81.

de Hoog, G. & Rao, V. (1975). Some new hyphomycetes. *Persoonia* **8**, 207-212.

Dingley, J. (1951). The Hypocreales of New Zealand II. The genus *Nectria*. *Transactions of the Royal Society of New Zealand* **79**, 177-202.

Dingley, J. (1953). The Hypocreales of New Zealand V. The genera *Cordyceps* and *Torrubiella*. *Transactions of the Royal Society of New Zealand* **81**, 329-343.

Dingley, J. (1954). The Hypocreales of New Zealand.VI. The Genera *Hypocrella*, *Barya*, *Claviceps* and *Podonectria*. *Transactions of the Royal Society of New Zealand* **81**, 489-499.

Domsch, K., Gams, W. & Anderson, T. (1980). *Compendium of soil fungi*. Academic Press: London.

Dromph, K. & Vestergaard, S. (2002). Pathogenicity and attractiveness of entomopathogenic hyphomycete fungi to collembolans. *Applied Soil Ecology* **21**, 197-210.

Edwards, G. (1764). *Gleanings of natural history, containing figures of quadrupeds, birds, insects, plants, etc. Vol. 3*. Royal College of Physicians: London.

Edwards, J.S. (1965). Entomogenous micro-organisms of *Prionoplus reticularis* (White). *New Zealand Entomologist* **3**, 21.

Eilenberg, J., Hajek, A. & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl* **46**, 387-400.

Ekesi, S., Shah, P., Clark, S. & Pell, J. (2005). Conservation biological control with the fungal pathogen *Pandora neoaphidis*; implications of aphid species, host plant and predator foraging. *Agricultural and Forest Entomology* 21-30.

Enkerli, J., Widmer, F., Gessler, C. & Keller, S. (2001). Strain-specific microsatellite markers in the entomopathogenic fungus *Beauveria brongniartii*. *Mycological Research* **105**, 1079-1087.

Estrada, M., Camacho, M. & Benito, C. (2007). The molecular diversity of different isolates of *Beauveria bassiana* (Bals.) Vuill. as assessed using intermicrosatellites (ISSRs). *Cellular & Molecular Biology Letters* **12**, 240-252.

Evans, H.C. (1974). Natural control of arthropods, with special reference to ants (Formicidae), by fungi in tropical high forest of Ghana. *Journal of Applied Ecology* **11**, 37-49.

Evans, H.C. (1982). Entomogenous fungi in tropical forest ecosystems - an appraisal. *Ecological Entomology* **7**, 47-60.

Evans, H.C. (1988). Coevolution of entomogenous fungi and their insect hosts. In *Coevolution of Fungi with Plants and Animals* (eds. K. Pirozynski & D. Hawksworth) pp. 149-171. Academic Press: London.

Evans, H.C. (1989). Mycopathogens of insects in epigeal and aerial habitats. In *Insect-fungus interactions* (eds. N. Wilding, N. Collins, P. Hammond & J. Webber) pp. 205-237. Academic Press: London.

Evans, H.C. & Samson, R.A. (1982a). *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems I. The Cephalotes (Myrmicinae) complex. *Transactions of the British Mycological Society* **79**, 431-453.

Evans, H.C. & Samson, R.A. (1982b). Entomogenous fungi from the Galapagos islands. *Canadian Journal of Botany* **60**, 2325-2333.

Evans, H.C. & Samson, R.A. (1984). *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems II. The *Camponotus* (Formicinae) complex. *Transactions of the British Mycological Society* **81**, 127-150.

Fargues, J. & Remaudiere, G. (1977). Considerations on the specificity of entomopathogenic fungi. *Mycopathologia* **62**, 31-37.

Federici, B. (1999). Naturally occuring baculoviruses for insect pest control. In *Biopesticides: use and delivery* (eds. F. Hall & J. Menn) pp. 301-320.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783-791.

Ferguson, A.M. & Fletcher, J.D. (1991). Greedy scale and the fungus *Fusarium stilboides*. *Proceedings of the New Zealand Weed and Pest Control Conference* **44**, 260-161.

Fraser, K. (1994). John Hill and the Royal Society in the Eighteenth Century. *Notes and Records of the Royal Society of London* **48**, 43-67.

Freimoser, F., Hu, G. & St Leger, R.J. (2005). Variation in gene expression patterns as the insect pathogen *Metarhizium anisopliae* adapts to different host cuticles or nutrient deprivation in vitro. *Microbiology* **151**, 361-371.

Fukatsu, T., Sato, H. & Kuriyama, H. (1997). Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*. *Journal of Invertebrate Pathology* **70**, 203-208.

Fuller, R., Buchanan, P. & Roberts, M. (2004). Maori Knowledge of Fungi / Matauranga o Nga Horere. In *Introduction to Fungi of New Zealand* (ed. E. McKenzie) pp. 81-118. Fungal Diversity Press: Hong Kong.

Fuxa, J. (1987). Ecological considerations for the use of entomopathogens in IPM. *Annual Review of Entomology* **32**, 225-251.

Gams, W. (1971). *Cephalosporium-artige Schimmelpilze*. Gustav Fischer Verlag: Stuttgart.

Gams, W., Hodge, K.T., Samson, R.A., Korf, R.P. & Seifert, K.A. (2005). (1684) Proposal to conserve the name *Isaria* (anamorphic fungi) with a conserved type. *Taxon* **54**, 537-539.

Gams, W., O'Donnell, K., Schroers, H.-J. & Christensen, M. (1998). Generic classification of some more hyphomycetes with solitary conidia borne on phialides. *Canadian Journal of Botany* **76**, 1570-1583.

Geiser, D. (2004). Practical molecular taxonomy of fungi. In *Advances in fungal biotechnology for industry, agriculture and medicine* (eds. J. Tkacz & L. Lange) pp. 3-14. Kluwer Academic/Plenum Publishers: New York

Geiser, D., del Mar Jiminez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T., Zhang, N., Kuldau, G. & O'Donnell, K. (2004). FUSARIUM-ID v. 1.0: A DNA Sequence Database for Identifying *Fusarium*. *European Journal of Plant Pathology* **110**, 473-479.

Gillespie, J., Bailey, A., Cobb, B. & Vilcinskas, A. (2000). Fungi as elicitors of insect immune responses. *Archives of Insect Biochemistry and Physiology* **44**, 49-68.

Glare, T. (2004). Molecular characterisation in the entomopathogenic fungal genus *Beauveria*. *Laimburg Journal* **1**, 286-298.

Glare, T.R. & Inwood, A.J. (1998). Morphological and genetic characterisation of *Beauveria* spp. from New Zealand. *Mycological Research* **102**, 250-256.

Glare, T.R., Inwood, A.J., van der Wielen, P. & Nelson, T. (1996a). Identification of novel starins of Beauveria and Metarhizium in New Zealand. In *Proceedings of the Third International Workshop on Microbial Control of Soil Dwelling Pests* (eds. T.A. Jackson & T.R. Glare) pp. 39-49.

Glare, T.R., Jackson, T.A. & Cisternas, E. (1993a). *Beauveria vermiconia* is an entomopathogenic fungus. *Mycological Research* **97**, 336-338.

Glare, T.R., Milner, R.J. & Beaton, C.D. (1996b). Variation in *Metarhizium*, a genus of fungal pathogens attacking Orthoptera: is phialide morphology a useful taxonomic criterion? *Journal of Orthopteran Research* **5**, 19-27.

Glare, T., O'Callaghan, M. & Wigley, P.J. (1993b). Checklist of naturally-occurring entomopathogenic microbes and nematodes in New Zealand. *New Zealand Journal of Zoology* **20**, 95-120.

Glare, T.R., Reay, S.D., Nelson, T.L. & Moore, R. (2008). *Beauveria caledonica* is a naturally occurring pathogen of forest beetles. *Mycological Research* **112**, 352-360.

Glass, N.L. & Donaldson, G.C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and environmental microbiology* **61**, 1323-1330.

Goettel, M.S. & Inglis, G.D. (1997). Fungi: Hyphomycetes. In *Manual of Techniques in Insect Pathology* (eds. L.A. Lacey) pp. 213-249. Academic Press: San Diego.

Goettel, M.S., Poprawski, T., Vandenberg, J.D., Li, Z. & Roberts, D.W. (1990). Safety to nontarget invertebrates of fungal biocontrol agents. In *Safety of microbial insecticides* (eds. L. Marshall & L.A. Lacey) pp. 209-231. CRC Press: Boca Raton.

Goh, H.H., Barker, G.M., Addison, P.J., Lyons, S.N. & Firth, A.C. (1991a). Comparative pathogenicity of *Beauveria bassiana* isolates to adult Argentine stem weevil in the laboratory. *Proceedings of the New Zealand Weed and Pest Control Conference* **44**, 185-188.

Goh, H.H., Willoughby, B., Prestidge, R.A. & Lyons, S.N. (1991b). Pathogenicity of *Beauveria*, *Metarhizium*, *Paecilomyces* and *Fusarium* isolates against early instar grass grub (*Costelytra zealandica*) larvae. *Proceedings of the NZ Weed and Pest Control Conference* **44**, 209-211.

Goldson, S., Rowarth, J. & Caradus, J. (2005). The impact of invasive invertebrate pests in pastoral agriculture: a review. *New Zealand Journal of Agricultural Research* **48**, 401-415.

Gray, G. (1858). *Notices of insects that are known to form the bases of fungoid parasites.* Privately printed: London.

Grehan, J.R. (1982). Infection of *Aenetus virescens* (Lepidoptera: Hepialidae) larvae by the fungus Beauveria bassiana. *New Zealand Entomologist* **7**, 327-329.

Grehan, J.R. & Wigley, P.J. (1984). Fungal and bacterial diseases of puriri moth, *Aenetus virescens* (Lepidoptera: Hepialidae), larvae. *New Zealand Entomologist* **8**, 61-63.

Hadapad, A., Reineke, A. & Zebitz, C.P.W. (2006). Genetic variability among *Beauveria brongniartii* (Saccardo) Petch isolates from various geographical and host origins based on AFLP analysis. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 15, 71-76.

Hajek, A.E. (1997). Ecology of terrestrial fungal entomopathogens. *Advances in Microbial Ecology* **15**, 193-249.

Hajek, A.E. (2004). *Natural enemies: an introduction to biological control.* Cambridge University Press: New York.

Hajek, A.E., Delalibera Jùnior, I. & McManus, M.L. (2007a). Introduction of exotic pathogens and documentation of their establishment and impact. In *Field manual of techniques in invertebrate pathology* (eds. L.A. Lacey & H.K. Kaya) pp. 299-325. Springer: Dordrecht.

Hajek, A.E. & Goettel, M.S. (2007). Guidelines for evaluating effects of entomopathogens on non-target organisms. In *Field manual of techniques in invertebrate pathology* (eds. L.A. Lacey & H.K. Kaya) Springer: Dordrecht.

Hajek, A.E., McManus, M.L. & Delalibera Jùnior, I. (2007b). A review of introductions of pathogens and nematodes for classical biological control of insects and mites. *Biological Control* **41**, 1-13.

Hajek, A.E. & St Leger, R.A. (1994) Interactions between fungal pathogens and their hosts. *Annual review of Entomology* **39**, 293-322.

Harney, S. & Widden, P. (1991a). Physiological properties of the entomopathogenic hyphomycete *Paecilomyces farinosus* in relation to its role in the forest ecosystem. *Canadian Journal of Botany* **69**, 1-5.

Harney, S. & Widden, P. (1991b). The ecology of *Paecilomyces farinosus* in two balsam fir forests infested with spruce budworm. *Canadian Journal of Botany* **69**, 512-515.

Harris, R.J. & Etheridge, N. (2001). Comparison of baits containing fipronil and sulfluramid for the control of *Vespula* wasps. *New Zealand Journal of Zoology* **28**, 39-48.

Harris, R.J., Harcourt, S.J., Glare, T.R., Rose, E. & Nelson, T.J. (2000). Susceptibility of *Vespula vulgaris* (Hymenoptera : Vespidae) to generalist entomopathogenic fungi and their potential for wasp control. *Journal of Invertebrate Pathology* **75**, 251-258.

Harris, R.J. & Rees, J.S. (2000). Aerial poisoning of wasps. *Science for Conservation* **162**, 1-26.

Hegedus, D. & Khachatourians, G. (1996). Identification and differentiation of the entomopathogenic fungus *Beauveria bassiana* using polymerase chain reaction and single-strand conformation polymorphism analysis. *Journal of Invertebrate Pathology* **67**, 289-299.

Helson, G. (1965). Entomogenous microorganisms of New Zealand. *Transactions of the Royal Society of New Zealand Zoology* **6**, 1-6.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J, Redhead, S., Schoch, C.S., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Catherine Aime, M.,Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P., Yu-Cheng Dai, P.W., Gams, W., Geiser, Griffith, G.W., Gueidan, C. C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.D, Ljalg, U.K., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Dlikowska, J.M., Miller, A., Moncalvo, Mozley-Standridge, J., Oberwinkler, F., Parmasto, Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Paulo Sampaio, J., Schubler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A, Walker, C., Wang, Z., Weir, W., Weiss, M., White, M.M.,Winka, K., Yao, Y. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**, 509-547.

Hill, R., Cumber, R. & Allan, D. (1985). Parasitoids introduced to attack larvae of the Noctuidae (Lepidoptera) and their establishment in New Zealand, 1968-1978. *DSIR Entomology Division Report* **5**, 1-24.

Hodge, K.T. (1998). Revisionary studies in *Hirsutella (*Anamorphic Hypocreales: Clavicipitaceae). Unpublished Ph.D. thesis, Cornell University, Ithaca.

Hodge, K.T., Gams, W., Samson, R.A., Korf, R.P. & Seifert, K.A. (2005). Lectotypification and status of *Isaria* Pers. : Fr. *Taxon* **54**, 485-489.

Hokkanen, H.M.T., Bigler, F., Burgio, G., van Lenteren, J.C. & Thomas, M.B. (2003). Ecological risk assesment framework for biological control. In *Environmental impacts of microbial insecticides: need and methods for risk assessment.* (eds. H.M.T. Hokkanen & A.E. Hajek) pp. 1-14. Kluwer Academic Publishers: Dordrecht.

Holder, D. & Keyani, N. (2005). Adhesion of the entomopathogenic fungus *Beauveria (Cordyceps) bassiana* to substrata. *Applied and Environmental Microbiology* **71**, 5260-5266.

Holloway, B. (1956). Revision of the New Zealand pill millipedes (Oniscomorpha, Sphaerotheridae). *Transactions of the Royal Society of New Zealand* **84**, 431-446.

Hooker, W. (1837). *Icones Plantarum Series I: Volumes I-V.* Longman, Rees, Orme, Brown, Green and Longman: London.

Huang, B., Li, C.R., Li, Z.G., Fan, M.Z. & Li, Z.Z. (2002). Molecular identification of the teleomorph of *Beauveria bassiana*. *Mycotaxon* **81**, 229-236.

Huelsenbeck, J. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754-755.

Hughes, S. (1951). Studies on micro-fungi. XI. Some Hyphomycetes, which produce phialides. *Mycological Papers* **45**, 1-36

Hughes, S. (1953). Conidiophores, conidia, and classification. *Canadian Journal of Botany* **31**, 577-659.

Humber, R.A. (2000). Fungal pathogens and parasites of insects. In *Applied Microbial Systematics* (eds. F. Preist & M. Goodfellow) pp. 203-230. Kluwer Academic Publishers: Dordrecht.

Humber, R.A. (2008). Evolution of entomopathogenicity in fungi. *Journal of Invertebrate Pathology* ***98, 262-266.***

Hummel, R., Walgenbach, J., Barbercheck, M., Hoyt, G. & Arellano, C. (2002). Effects of production practices on soil-borne entomopathogens in western North Carolina vegetable systems. **31**, 84-91.

Hywel-Jones, N. (1995a). *Cordyceps sphecocephala* and a *Hymenostilbe sp.* infecting wasps and bees in Thailand. *Mycological Research* **99**, 154- 158.

Hywel-Jones, N.L. (1995b). *Cordyceps brunneapunctata* sp. nov. infecting beetle larvae in Thailand. *Mycological Research* **99**, 1195- 1198.

Hywel-Jones, N.L. (1996). *Cordyceps myrmecophila*-like fungi infecting ants in the leaf litter of tropical forest in Thailand. *Mycological Research* **100**, 613- 619.

Hywel-Jones, N. (1997a). The biological diversity of invertebrate pathogenic fungi. In *Biodiversity of tropical microfungi* (ed. K. Hyde) pp. 107-119. Hong Kong University Press: Hong Kong.

Hywel-Jones, N. (1997b). *Torrubiella petchii*, a new species of scale insect pathogen from Thailand. *Mycological Research* **101**, 143-145.

Hywel-Jones, N. (2001). A review of invertebrate pathogenic Clavicipitaceae from Thailand. *BRT Research Reports* **2001**, 34-41.

Hywel-Jones, N. (2002). The importance of invertebrate-pathogenic fungi from the tropics. In *Tropical Mycology, Vol.2, Micromycetes* (eds. R. Watling, J.C. Frankland, A.M. Ainsworth, S. Isaac & C.H. Robinson) pp. 133-144. CAB International: Wallingford.

Hywel-Jones, N. (2004). The relationship between the entomopathogenic fungal genera *Cordyceps* and *Beauveria*. *Laimburg Journal* **1**, 299-304.

Hywel-Jones, N.L. & Sivichai, S. (1995). *Cordyceps cylindrica* and its association with Nomuraea atypicola in Thailand. *Mycological Research* **99**, 809- 812.

Inglis, G.D., M.S., G., Butt, T.M. & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In *Fungi as biocontrol agents: progress, problems and potential* (eds. T.M. Butt, C. Jackson & N. Magan) pp. 23-69. CAB International: Wallingford.

Inglis, P. & Tigano-Milani, M. (2006). Identification and taxonomy of some entomopathogenic *Paecilomyces* spp. (Ascomycota) isolates using rDNA-ITS Sequences. *Genetics and Molecular Biology* **29**, 132-136.

Isaka, M., Kittakoop, P., Kirtikara, K., Hywel-Jones, N.L. & Thebtaranonth, Y. (2005). Bioactive substances from insect pathogenic fungi. *Accounts of Chemical Research* **38**, 813-823.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schubler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W. & Vilgalys, R. (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**, 818-822.

Jarrold, S., Moore, D., Potter, U. & Charnley, A. (2007). The contribution of surface waxes to pre-penetration growth of an insect fungus on host cuticle. *Mycological Research* **111**, 240-249.

Johnson, D., Sung, G.H., Hywel-Jones, N., Luangsa-Ard, J.J., Bischoff, J.F., Kepler, R. & Spatafora, J.W. (2009). Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). *Mycological Research* **113**, 279-289.

Kadlec, Z., Simek, P., Heydova, A., Jegorov, A., Matha, V., Landa, Z. & Eyal, J. (1994). Chemotaxonomic discrimination among the fungal genera *Tolypocladium*, *Beauveria* and *Paecilomyces*. *Biochemical Systematics and Ecology* **22**, 803-808.

Kaya, H.K. & Lacey, L.A. (2007). Introduction to microbial control. In *Field manual of techniques in invertebrate pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests* (eds. L.A. Lacey & H.K. Kaya) pp. 3-7. Springer: Dordrecht.

Keller, S. & Zimmermann, G. (1989). Mycopathogens of soil insects. In *Insect-fungus interactions* (eds. N. Wilding, N. Collins, P. Hammond & J. Webber) pp. 239-270. Academic Press: London.

Kerwin, J. & Washino, R. (1986). Cuticular regulation of host recognition and spore germination by entomopathogenic fungi. In *Fundamental and applied aspects of invertebrate pathology* (eds. R.A. Samson, J. Vlak & D. Peters) pp. 423-425. Ponsen and Looijen: Wageningen.

Kikuchi, H., Takahashi, N. & Oshima, Y. (2004). Novel aromatics bearing 4-O-methylglucose unit isolated from the oriental crude drug *Bombyx batryticatus*. *Tetrahedron Letters* **45**, 367-370.

Kirby, W. (1826). Diseases of insects. In *An introduction to entomology or elements of the natural history of insects* (eds. W. Kirby & W. Spence) pp. 197-232. Longman, Rees, Orme, Brown and Green: London.

Kirk, T.W. (1909). Biology division. Report of T.W. Kirk, F.L.S., biologist. In *Report of the Department of Agriculture of New Zealand* (ed. anon.) pp. 44-89.

Kirschner, R. (2001). Diversity of filamentous fungi in bark beetle galleries in Central Europe. In *Trichomycetes and other fungal groups* (eds. R. Lichtwardt, J. Misra & B. Horn) pp. 175-196. Science publishers: Enfield.

Kobayasi, Y. (1939). On the genus *Cordyceps* and its allies on cicadae from Japan. *Bulletin of the Biogeographical Society of Japan* **9**, 145-176.

Kobayasi, Y. (1941). The genus *Cordyceps* and its allies. *Science Report of the Tokyo Bunrika Daigaku, Section B. No. 84* **5**, 53-260.

Kobayasi, Y. (1982). Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. *Transactions of the Mycological Society of Japan* **23**, 329-364.

Kobayasi, Y. & Shimizu, D. (1963). Monographic studies of *Cordyceps* 2. Group parasitic on Cicadae. *Bulletin of the National Science Museum, Tokyo* **6**, 286-314.

Kobayasi, Y. & Shimizu, D. (1982). Monograph of the genus *Torrubiella*. *Bulletin of the National Science Museum, Tokyo, Series B* **8**, 43-78.

Kouvelis, V., Ghikas, D., Edgington, S., Typas, M. & Moore, D. (2008). Molecular characterization of isolates of *Beauveria bassiana* obtained from overwintering and summer populations of Sunn Pest *Eurygaster integriceps*. *Letters in Applied Microbiology* **46**, 414-420.

Kritsky, G. (2001). Periodical revolutions and the early history of the "locust" in American cicada terminology. *American Entomologist* **47**, 186-188.

Kurihara, Y., Sukarno, N., Ilyas, M., Yuniarti, E., Mangunwardoyo, W., Saraswati, R., Park,J.Y., Inaba, S., Widyastuti, Y. & Ando, K. (2008). Entomopathogenic fungi isolated from suspended-soil-inhabiting arthropods in East Kalimantan, Indonesia. *Mycoscience* **49**, 241-249.

Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P. (2001). Insect pathogens as biological control agents: Do they have a future? *Biological Control* **21**, 230-248.

Lacey, L.A. & Shapiro-Ilan, D. (2003). The potential role for microbial control of orchard insect pests in sustainable agriculture. *Food, Agriculture and Environment* **1**, 326-331.

Larivière, M.-C. (1995). Cydnidae, Acanthosomatidae, and Pentatomidae (Insecta: Heteroptera): systematics, geographical distribution, and bioecology. *Fauna of New Zealand* **35**, 1-112.

Larivière, M.-C. , Rhode, B. & Larochelle, A. (2006). New Zealand Cicadas (Hemiptera: Cicadidae): A virtual identification guide. The New Zealand Hemiptera Website, NZHW. http://hemiptera.landcareresearch.co.nz/.

Leatherdale, D. (1970). The arthropod hosts of entomogenous fungi in Britain. *Entomophaga* **15**, 419-435.

Li, Z. (1988). List on the insect hosts of *Beauveria bassiana*. In *Study and Application of Entomogenous Fungi in China, Vol. 1* (eds. Y. Li & Z. Li *et al.*) pp. 241-255. Academic Periodical Press: Beijing.

Li, Z.Z., Li, C.R., Huang, B. & Fan, M.Z. (2001). Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. *Chinese Science Bulletin* **46**, 751-753.

Liang, Z. (1981). Two new species of *Paecilomyces* from insects. *Acta Microbiologica Sinica* **21**, 31-34.

Liang, Z., Han, Y., Chu, H. & Liu, A.Y. (2005). Studies on the genus *Paecilomyces* in China I. *Fungal Diversity* **20**, 83-101.

Liang, Z., Wang, B. & Kang, J.C. (2003). Several rare entomopathogenic fungi from the Western Sichaun mountains. *Fungal Diversity* **12**, 129-34.

Lieckfeldt, E. & Seifert, K. (2000). An evaluation of the use of ITS sequences in the taxonomy of the Hypocreales. *Studies in Mycology* **45**, 35-44.

Liu, Z., Liang, Z., Liu, A.Y., Yao, Y.J., Hyde, K.D. & Yu, Z. (2002). Molecular evidence for teleomorph-anamorph connections in *Cordyceps* based on ITS-5.8S rDNA sequences. *Mycological Research* **106**, 1100-1108.

Lloyd, C. (1915). Mycological notes, no 39. *Mycological Writings* **4**, 525-540.

Lloyd, C. (1919). Mycological notes, no. 61. *Mycological Writings* **5**, 877-903.

Lloyd, C. (1920). Mycological notes, no. 62. *Mycological Writings* **6**, 904-944.

Lloyd, C. (1923). Mycological notes, no. 68. *Mycological Writings* **7**, 1169-1184.

Lockwood, J. (1993a). Environmental issues involved in biological control of rangeland grasshoppers (Orthoptera: Acrididae) with exotic agents. *Environmental Entomology* **22**, 503-518.

Lockwood, J. (1993b). Benefits and costs of controlling rangeland grasshoppers (Orthoptera: Acrididae) with exotic organisms: Search for a null hypothesis and regulatory compromise. *Environmental Entomology* **22**, 904-914.

Luangsa-ard, J.J. (2004). A phylogenetic study of *Paecilomyces* and related genera. Unpublished Ph.D. thesis. Kasetsart University, Bangkok.

Luangsa-ard, J.J., Hywel-Jones, N.L., Manoch, L. & Samson, R.A. (2005). On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycological Research* **109**, 581-589.

Luangsa-ard, J.J., Hywel-Jones, N. & Samson, R. (2004). The polyphyletic nature of *Paecilomyces* sensu lato based on 18S-generated rDNA phylogeny. *Mycologia* **96**, 773-780.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V. & Schoch, Arnold, A.E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G-H., Lucking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y-W.,

Matheny, P.B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R. & Vilgalys, R. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**, 1446-1480.

Luz, C., Tigano, M.S., Silva, I.G., Cordeiro, C.M. & Aljanabi, S.M. (1998). Selection of *Beauveria bassiana* and *Metarhizium anisopliae* Isolates to Control *Triatoma infestans*. *Memorias do Instituto Oswaldo Cruz* **93**, 839-846.

MacLeod, D.M. (1954). Investigations on the genera *Beauveria* Vuill. and *Tritirachium* Limber. *Canadian Journal of Botany* **32**, 818-890.

Mains, E. (1940). Species of *Cordyceps*. *Mycologia* **32**, 310-320.

Mains, E. (1947). New and interesting species of *Cordyceps*. *Mycologia* **39**, 535-545

Mains, E. (1949). New species of *Torrubiella*, *Hirsutella* and *Gibellula*. *Mycologia* **41**, 304-310.

Mains, E. (1950). Entomogenous species of *Akanthomyces*, *Hymenostilbe* and *Insecticola* in North America. *Mycologia* **42**, 567-589.

Mains, E. (1955). Some entomogenous species of *Isaria*. *Papers of the Michigan Academy of Science, Arts, and Letters* **40**, 23-32.

Major, R. (1944). Agostino Bassi and the parasitic history of disease. *Bulletin of the History of Medicine* **16**, 97-107.

Manocha, M.S. & Chen, Y. (1990). Specificity of attachment of fungal parasites to their hosts. *Canadian Journal of Microbiology* **36**, 69-76.

Marshall, R.K., Lester, M.T., Glare, T.R. & Christeller, J.T. (2003). The fungus, *Lecanicillium muscarium*, is an entomopathogen of passionvine hopper (*Scolypopa australis*). *New Zealand Journal of Crop and Horticultural Science* **31**, 1-7.

Massee, G. (1895). A revison of the genus *Cordyceps*. *Annals of Botany* **9**, 1-44.

Matheny, P.B., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Nilsson, R.H., Hughes, K.W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., Langer, E., Langer, G., McLaughlin, D.J., Wilson, A.W., Froslev, T., Ge, Z.W., Kerrigan, R.W., Slot J. C., Yang Z.-L., Baroni T. J., Fischer M., Hosaka K., Matsuura K., Seidl M. T., Vauras J. & Hibbett D. S. (2007). Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* **43**, 430-451.

Maurer, P., Couteaudier, Y., Girard, P.A., Bridge, P.D. & Riba, G. (1997). Genetic diversity of *Beauveria bassiana* and relatedness to host insect range. *Mycological Research* **101**, 159-164.

McCoy, C. & Boucias, D.G. (1989). Selection of *Beauveria bassiana* pathotypes as potential microbial control agents of soil-inhabiting citrus weevils. *Memorias do Instituto Oswaldo Cruz* **84**, 75-80.

McDonald, B.A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology* **87**, 448-453.

McDowall, R. (2007). Process and pattern in the biogeography of New Zealand. *Journal of Biogeography* **35**, 197-212.

McKenzie, E.H.C. (2000). *Uromyces transversalis*, rust fungus infecting Iridaceae in New Zealand. *New Zealand Journal of Crop and Horticultural Science* **28**, 289-292.

Meyling, N.V. (2008). PCR-based characterisation of entomopathogenic fungi for ecological studies. *Deliverable 5.2 from VegQure. (http://orgprints/14345/1/DS_2_final.pdf)* 1-14.

Meyling, N.V. & Eilenberg, J. (2006). Occurrence and distribution of soilborne entomopathogenic fungi within a single organic agroecosystem. *Agriculture, Ecosystems and Environment* **113**, 341.

Meyling, N.V. & Eilenberg, J. (2007). Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control* **43**, 145-155.

Meyling, N.V., Pell, J.K. & Eilenberg, J. (2006). Dispersal of *Beauveria bassiana* by the activity of nettle insects. *Journal of Invertebrate Pathology* **93**, 121-126.

Micales, J., Bonde, M. & Peterson, G. (1986). The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxon* **27**, 405-449.

Milner, R.J. (1997). Prospects for biopesticides for aphid control. *Entomophaga* **42**, 227-239.

Milner, R.J., Soper, R. & Lutton, G. (1982). Field release of an Israeli strain of the fungus *Zoophthora radicans* (Brefeld) Batko for the biological control of *Theiroaphis trifolii* (Monell) *f. maculata*. *Journal of the Australian Entomological Society* **21**, 113-118.

Minter, D., Brady, B. & Hall, R. (1983). Five hyphomycetes isolated from eriophyid mites. *Transactions of the British Mycological Society* **81**, 455-471.

Mitchill, S. (1827). Views of the process in nature, by which, under particular circumstances, vegetables grow on the bodies of living animals. *The American Journal of Science and Arts* **12**, 21-28.

Moncalvo, J. & Buchanan, P.K. (2008). Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycological Research* **112**, 425-436.

Moyersoen, B., Beever, R.E. & Martin, F. (2003). Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist* **160**, 569-579.

Mugnai, L., Bridge, P.D. & Evans, H.C. (1989). A chemotaxonomic evaluation of the genus *Beauveria*. *Mycological Research* **92**, 199-209.

Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press: New York.

Nelson, T.L., Willoughby, B.E., Wilson, D., Eden, T. & Glare, T.R. (2004). Establishing the fungus *Beauveria bassiana* in pasture for clover root weevil *(Sitona lepidus)* control. *New Zealand Plant Protection* **57**, 314-318.

Neuveglise, C., Brygoo, Y., Vercambre, B. & Riba, G. (1994). Comparative analysis of molecular and biological characteristics of strains of *Beauveria brongniartii* isolated from insects. *Mycological Research* **98**, 322-328.

Nikoh, N. & Fukatsu, T. (2000). Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. *Molecular Biology and Evolution* **17**, 629-638.

Nikoh, N. & Fukatsu, T. (2001). Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of endoparasitic fungi of the genus *Cordyceps*. *Molecular Biology and Evolution* **18**, 1631–1642.

Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K. (2008). Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* **4**, 193-201.

Norup, T., Berg, T., Stenholm, H., Andersen, S. & Hojrup, P. (1996). Purification and characterization of five cuticular proteins from the spider *Araneus diadematus*. *Insect Biochemistry and Molecular Biology* **26**, 907-915.

Nylander, J. A. A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

O'Donnell, K. & Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**, 103-116.

Oborník, M., Jirku, M. & Dolezel, D. (2001). Phylogeny of mitosporic entomopathogenic fungi: is the genus *Paecilomyces* polyphyletic? *Canadian Journal of Microbiology* **47**, 813-819.

Onions, A. H. S. & Barron, G.L. (1967). Monophialidic species of *Paecilomyces*. *Mycological Papers*. **107**, 1-25.

Owen, M. (1919). The skin spot disease of potato tubers (*Oospora pustulans*.). *Bulletin of Miscellaneous Information (Royal Gardens, Kew)* **8**, 289-301.

Pacioni, G. & Frizzi, G. (1978). *Paecilomyces farinosus*, the conidial state of *Cordyceps memorabilis*. *Canadian Journal of Botany* **56**, 391-394.

Payne, C., Hafez, M., Jones, K., Harpaz, I., Akingbohungbe, A., Paterson, R. & Hag Amed, S. (1988). Pathogens for the control of insects: where next? [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **318**, 225-248.

Pearson, W. (1989). *Coleophora frischella* L., whitetipped clover casebearer , *C. spissicornis* Haworth, banded clover casebearer (Lepidoptera: Coleophoridae). In *A Review of biological control of invertebrate pests and weeds in New Zealand 1874-1987* (eds. P. J. Cameron, R.L. Hill, J. Bain & W.P. Thomas) pp. 73-85. CAB International: Wallingford.

Pell, J.K., Eilenberg, J., Hajek, A. & Steinkraus, D. (2001). Biology, ecology and pest management potential of Entomophthorales. In *Fungi as biocontrol agents: progress,*

problems and potential (eds. T.M. Butt, C. Jackson & N. Magan) pp. 71-167. CAB International: Wallingford.

Pemberton, R. (1999). Insects and other arthropods used as drugs in Korean traditional medicine. *Journal of Ethnopharmacology* **65**, 207-216.

Pennycook, S.R. & Galloway, D.J. (2004). Checklist of New Zealand "Fungi". In *Introduction to fungi of New Zealand* (eds. E.H.C. McKenzie) pp. 401-488. Fungal Diversity Press: Hong Kong.

Perrie, L. & Brownsey, P. (2007). Molecular evidence for long-distance dispersal in the New Zealand pteridophyte flora. *Journal of Biogeography* **34**, 2028-2038.

Petch, T. (1924). Studies in entomogenous fungi. IV. Some Ceylon *Cordyceps*. *Transactions of the British Mycological Society* **10**, 28-45.

Petch, T. (1926). Studies in entomogenous fungi. VIII. Notes on *Beauveria*. *Transactions of the British Mycological Society* **10**, 244-271Petch, T. (1931). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **16**, 55-76.

Petch, T. (1931). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **16**, 55-76.

Petch, T. (1932a). British species of *Hirsutella*. *The Naturalist* **1932**, 45-49.

Petch, T. (1932b). *Gibellula*. *Annales Mycologici* **30**, 386-393.

Petch, T. (1933). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **18**, 48-75.

Petch, T. (1934). *Isaria*. *Transactions of the British Mycological Society* **19**, 34-38.

Petch, T. (1936). *Cordyceps militaris* and *Isaria farinosa*. *Transactions of the British Mycological Society* **20**, 216-224.

Petch, T. (1937). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **21**, 34-67.

Piatti, P., Cravanzola, F., Bridge, P.D. & Ozino, O.I. (1998) Molecular characterization of *Beauveria brongniartii* isolates obtained from *Melolontha melolontha* in Valle d'Aosta (Italy) by RAPD-PCR. *Letters in Applied Microbiology* **26**, 317-324.

Pillai, J. & Smith, J. (1968). Fungal Pathogens of Mosquitoes in New Zealand 1. *Coelomomyces opifexi* sp. n., on the Mosquito *Opifex fuscus* Hutton. *Journal of Invertebrate Pathology* **11**, 316-320.

Pitt, J.I. & Hocking, A. (1985). Interfaces among genera related to *Aspergillus* and *Penicillium*. *Mycologia* **77**, 810-824.

Poprawski, T., Riba, G., Jones, W. & Aioun, A. (1988). Variation in isoesterase profiles of geographical populations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolated from *Sitona* weevils (Coleoptera: Curculionidae). *Environmental Entomology* **17**, 275-279.

Prior, C. (1992). Discovery and characterization of fungal pathogens for locust and grasshopper control. In *Biological control of locusts and grasshoppers* (eds. C. Lomer & C. Prior) pp. 159-180. CAB International: Wallingford.

Rakotonirainy, M.S., Dutertre, M., Brygoo, Y. & Riba, G. (1991). rRNA sequence comparison of *Beauveria bassiana*, *Tolypocladium cylindrosporum*, and *Tolypocladium extinguens*. *Journal of Invertebrate Pathology* **57**, 17-22.

Ramsbottom, J. (1941). The expanding knowledge of mycology since Linnaeus. *Proceedings of the Linnean Society of London* **151**, 280-367.

Rath, A.C., Carr, C.J. & Graham, B.R. (1995). Characterization of *Metarhizium anisopliae* strains by carbohydrate utilization (API50CH). *Journal of Invertebrate Pathology* **65**, 152- 161.

Reay, S.D., Brownbridge, M., Cummings, N.J., Nelson, T.L., Souffre, B., Lignon, C. & Glare, T.R. (2008). Isolation and characterization of *Beauveria* spp. associated with exotic bark beetles in New Zealand *Pinus radiata* plantation forests. *Biological Control* **46**, 484-494.

Reay, S., Hachet,C., Nelson, T.L., Brownbridge, M. & Glare, T. (2007). Persistence of conidia and potential efficacy of *Beauveria bassiana* against pinhole borers in New Zealand southern beech forests. *Forest Ecology and Management* **246**, 232-239.

Reeb, V, Lutzoni, F. & Roux, C. (2003). Contribution of RPB2 to multilocus phylogenetic studies of the Pezizomycotina (Euascomycetes, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**, 1036-1060.

Rehner, S.A. (2005). Phylogenetics of the insect pathogenic genus *Beauveria*. In *Insect-fungal associations: ecology and evolution* (eds. F.E. Vega & M. Blackwell) pp. 3-27. Oxford University Press: New York.

Rehner, S.A. (2009) Molecular systematics of entomopathogenic fungi. *In Insect pathogens: molecular approaches and techniques* (eds. S.P. Stock, J. Vandenberg, I Glazer, N. Boemare) pp. 145-158. CABI international, Wallingford.

Rehner, S.A., Aquino de Muro, M.A. & Bischoff, J.F. (2006a). Description and phylogenetic placement of *Beauveria malawiensis* sp. nov. (Clavicipitaceae, Hypocreales). *Mycotaxon* **98**, 137-145.

Rehner, S.A. & Buckley, E.P. (2003). Isolation and characterization of microsatellite loci from the entomopathogenic fungus *Beauveria bassiana* (Ascomycota : Hypocreales). *Molecular Ecology Notes* **3**, 409-411.

Rehner, S.A. & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**, 84-98.

Rehner, S.A., Posada, F., Buckley, E.P., Infante, F., Castillo, A. & Vega, F.E. (2006b). Phylogenetic origins of African and Neotropical *Beauveria bassiana* s.l. pathogens of the coffee berry borer, *Hypothenemus hampei*. *Journal of Invertebrate Pathology* **93**, 11-21.

Renker, C., Otto, P., Schneider, K., Zimdars, B., Maraun, M. & Buscot, F. (2005). Oribatid mites as potential vectors for soil microfungi: study of mite-associated fungal species. *Microbial Ecology* **50**, 518-528.

Reynolds, D. (1993). The fungal holomorph: an overview. In *The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics* (eds. D. Reynolds & J. Taylor) pp. 15-25. CAB International: Wallingford.

Riley, M. (1994). *Maori Healing and Herbal. New Zealand Ethonobotanical Sourcebook.* Viking Sevenseas NZ: Paraparaumu.

Ronquist, F. & Huelsenbeck, J. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Rossi, W. &.Weir, A.(1998). *Triainomyces*, a new genus of Laboulbeniales on the pill-millipede, *Procyliosoma tuberculatum* from New Zealand. *Mycologia* **90**, 282-289.

Roy, H., Steinkraus, D., Eilenberg, J., Hajek, A. & Pell, J.K. (2006). Bizzare interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology* **51**, 331-357.

Samson, R.A. (1974). *Paecilomyces* and some allied hyphomycetes. *Studies in Mycology* **6**, 1-119.

Samson, R.A. (1995). Constraints associated with taxonomy of biocontrol fungi. *Canadian Journal of Botany* **73**, S83-S88.

Samson, R.A. & Evans, H. (1973). Entomogenous fungi from Ghana 1. The genera *Gibellula* and *Pseudogibellula. Acta Botanica Neerlandica* **22**, 522-528.

Samson, R.A. & Evans, H.C. (1974). Notes on entomogenous fungi from Ghana II. The genus *Akanthomyces. Acta Botanica Neerlandica* **23**, 28-35.

Samson, R.A. & Evans, H.C. (1975). Notes on entomogenous fungi from Ghana III. The genus *Hymenostilbe. Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences* **78**, 73-80.

Samson, R. & Evans, H. (1977). Notes on entomogenous fungi from Ghana IV. The genera *Paecilomyces* and *Nomuraea. Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences* **80**, 127-134.

Samson, R.A. & Evans, H.C. (1982). Two new *Beauveria* spp. from South America. *Journal of Invertebrate Pathology* **39**, 93-97

Samson, R.A., Evans, H.C. & Latge, J.P. (1988). *Atlas of entomopathogenic fungi.* Springer-Verlag: Berlin.

Samson, R.A., McCoy, C. & O'Donnell, K. (1980). Taxonomy of the acarine parasite *Hirsutella thompsonii. Mycologia* **72**, 359-377.

Samson, R.A. & Soares, G. (1984). Entomopathogenic species of the hyphomycete genus *Tolypocladium. Journal of Invertebrate Pathology* **43**, 133-139.

Sasaki, F., Miyamoto, T., Tamai, Y. & Yajima, T. (2007). Note on *Cordyceps brongniartii* Shimazu collected from the wild in Japan. *Mycoscience* **48**, 312-315.

Scott, R.R. (1975). A study of the biology and population dynamics of *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae) in Canterbury, New Zealand. Unpublished Ph.D. thesis, Lincoln University, Lincoln.

Seifert, K. & Crous, P. (2008). The all-fungi DNA barcoding campaign (FunBOL). *Persoonia* **20**, 106.

Seifert, K. & Gams, W. (2001). The taxonomy of anamorphic fungi. In *The Mycota VII. Part A.* (eds. D.J. McLaughlin, E.G. McLaughlin & P.A. Lemke) pp. 307-347. Springer-Verlag: Berlin.

Seifert, K.A., Wingfield, B.D. & Wingfeld, M.J. (1995). A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. **73**, S760-S767. Shah, P.A. & Pell, J.K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology* **61**, 413-423.

Shimazu, M. (2001). *Paecilomyces cateniannulatus*, a commonly found, but an unrecorded entomogenous fungus in Japan. *Applied Entomology and Zoology* **36**, 283-288.

Shimazu, M., Mitsuhashi, W. & Hashimoto, H. (1988). *Cordyceps brongniartii* sp. nov., the teleomorph of *Beauveria brongniartii*. *Transactions of the Mycological Society of Japan* **29**, 323-330.

Sierwald, P. & Bond, J. (2007). Current status of the myriapod class Diplopoda (millipedes): Taxonomic diversity and ecology. *Annual Review of Entomology* **52**, 401-420.

Sigler, L., Frances, S. & Panter, C. (1987). *Culicinomyces bisporalis*, a new entomopathogenic hyphomycete from larvae of the mosquito *Aedes kochi*. *Mycologia* **79**, 493-500.

Sitch, J. & Jackson, C. (1997). Pre-penetration events affecting host specificity of *Verticillium lecanii*. *Mycological Research* **101**, 535-541.

Smith, R. & Grula, E. (1981). Nutritional requirements for conidial germination and hyphal growth of *Beauveria bassiana*. *Journal of Invertebrate Pathology* **37**, 222-230.

Sogonov, M.V., Schroers, H.J., Gams, W., Dijksterhuis, J. & Summerbell, R.C. (2005). The hyphomycete *Teberdinia hygrophila* gen. nov., sp. nov. and related anamorphs of *Pseudeurotium* species. *Mycologia* **97**, 695-709.

Soper, R. (1974). The genus *Massospora*, entomopathogenic for cicadas, Part 1, taxonomy of the genus. *Mycotaxon* 13-40.

Sosnowska, D., Balazy, S., Prishchepa, L. & Mikulskaya, N. (2004). Biodiversity of arthropod pathogens in the Bialowieza Forest. *Journal of Plant Protection Research* **44**, 313-321.

Spatafora, J.W., Sung, G.H., Sung, J.M., Hywel-Jones, N.L. & White, W.J. (2007). Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology* **16**, 1701-1711.

Speare, A. (1920). On certain entomogenous fungi. *Mycologia* **12**, 62-76.

St Leger, R.J. (1991). Integument as a barrier to microbial infections. In *Physiology of the insect epidermis* (eds. K. Binnington & A. Retnakaran) pp. 285-306. CSIRO: Canberra.

St Leger, R.J. (1993). Biology and mechanisms of insect-cuticle invasion by Deuteromycete fungal pathogens. In *Parasites and pathogens of insects* (eds. N. Beckage, S. Thompson & B. Federici) pp. 211-229. Academic Press: San Diego.

St Leger, R.J. (1995). The role of cuticle-degrading proteases in fungal pathogenesis of insects. *Canadian Journal of Botany* **73**, S1119-S1125.

St Leger, R., Allee, L., May, B., Staples, R. & Roberts, D.W. (1992a). World-wide distribution of genetic variation among isolates of *Beauveria* spp. *Mycological Research* **96**, 1007-1015.

St Leger, R., Bidochka & Roberts, D.W. (1994). Germination triggers of *Metarhizium anisopliae* conidia are related to host species. *Microbiology* **140**, 1651-1660.

St Leger, R., Staples, R. & Roberts, D. (1992b). Genetic differences in allozymes and in formation of infection structures among isolates of the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* **60**, 89-101.

Steinhaus, E. (1956). Microbial control - the emergence of an idea. A brief history of insect pathology through the nineteenth century. *Hilgardia* **26**, 107-160.

Steinhaus, E. (1975). *Disease in a minor chord.* Ohio State University Press: Columbus.

Stensrud, O., Hywel-Jones, N.L. & Schumacher, T. (2005). Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly. *Mycological Research* **109**, 41-56.

Stolk, A.C. & Samson, R.A. (1972). The genus *Talaromyces*. Studies on *Talaromyces* and related genera II. *Studies in Mycology* **2,** 1-65.

Suh, S.O., Spatafora, J.W., Ochiel, G., Evans, H.C. & Blackwell, M. (1998). Molecular phylogenetic study of a termite pathogen *Cordycepioideus bisporus*. *Mycologia* **90**, 611-617.

Sung, G.H., Hywel-Jones, N.L., Sung, J.M., Luangsa-ard, J.J., Shrestha, B. & Spatafora, J.W. (2007a). Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 5-59.

Sung, G.H., Spatafora, J.W., Zare, R., Hodge, K.T. & Gams, W. (2001). A revision of *Verticillium* sect. Prostrata. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. *Nova Hedwigia* **72**, 29-46.

Sung, G.H., Sung, J.M., Hywel-Jones, N.L. & Spatafora, J.W. (2007b). A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* **44**, 1204-1223.

Sutton, B. (1996). Conidiogenesis, classification and correlation. In *A century of mycology* (ed. B. Sutton) pp. 137-160. Cambridge University Press: Port Chester.

Swofford, D. (2002). PAUP: Phylogenetic analysis using parsimony (and other methods), Version 4.0b10. Sinauer Associates: Sunderland.

Takatsuka, J. (2007). Characterization of *Beauveria bassiana* isolates from Japan using inter-simple-sequence-repeat-anchored polymerase chain reaction (ISSR-PCR) amplification. *Applied Entomology & Zoology* **42**, 563-571

Tamura, K., J., D., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.

Taylor, J. (1993). A contemporary view of the holomorph: nucleic acid sequence and computer databases are changing fungal classification. In *The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics* (eds. D. Reynolds & J. Taylor) pp. 3-13. CAB International: Wallingford.

Taylor, R. (1855). *Te ika a Maui; or, New Zealand and its inhabitants.*Wertheim & Macintosh: London. 490 pp.

Thomas, C., Moller, H., Plunkett, G. & Harris, R.J. (1990). The prevalence of introduced *Vespula vulgaris* wasps in a New Zealand beech forest community. *New Zealand Journal of Ecology* **13**, 63-72.

Todorova, S.I., Cote, J.C. & Coderre, D. (1998). Distinction between *Beauveria* and *Tolypocladium* by carbohydrate utilization. *Mycological Research* **102**, 81-87.

Toft, R.J. & Rees, J.S. (1998). Reducing predation of orb-web spiders (Araneidae) by controlling common wasps (*Vespula vulgaris*) in a New Zealand beech forest. *Ecological Entomology* **23**, 90-95.

Townsend, R.J., Glare, T.R. & Willoughby, B.E. (1995). The fungi *Beauveria* spp. cause epizootics in grass grub populations in Waikato. *Proceedings of the New Zealand Plant Protection Conference* **48**, 237-241.

Tulloch, M. (1976). The genus *Metarhizium*. *Transactions of the British Mycological Society* **66**, 407-411.

Uma Devi, K., Padmavathi, J., Uma Maheswara Rao, C., Ali P. Khan, A. & Mohan, C.M.. (2008). A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Science and Technology* **18**, 975-989.

Uma Devi, K., Reineke, A., Nageswara Rao Reddy, N., Uma Maheswara Rao, C. & Padmavathi, J. (2006). Genetic diversity, reproductive biology, and speciation in the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin. *Genome* **49**, 495-504.

Urtz, B.E. & Rice, W.C. (1997). RAPD-PCR characterization of *Beauveria bassiana* isolates from the rice water weevil *Lissorhoptrus oryzophilus*. *Letters in Applied Microbiology* **25**, 405-409.

Kouvelis, V.N., Ghikas, D.V., Edgington, S., Typas, M.A. & Moore, D. (2008). Molecular characterization of isolates of *Beauveria bassiana* obtained from overwintering and summer populations of Sunn Pest *Eurygaster integriceps*. *Letters in Applied Microbiology* **46**, 414-420.

Vanninen, I. (1996). Distribution and occurence of four entomopathogenic fungi in Finland. *Mycological Research* **100**, 93-101.

Vestergaard, S., Cherry, A., Keller, S. & Goettel, M.S. (2003). Safety of hyphomycete fungi as microbial control agents. In *Environmental impacts of microbial insecticides* (eds. H. Hokkanen & A. Hajek) pp. 35-62. Kluwer Academic Publishers: Dordrecht.

Vey, A., Fargues, J. & Robert, P. (1982). Histological and ultrastructural studies of factors determing the specificity of pathotypes of the fungus *Metarhizium anisopliae* for scarabeid larvae. *Entomophaga* **27**, 387-397.

von Arx, J. (1986). *Tolypocladium*, a synonym of *Beauveria*. *Mycotaxon* **25**, 153-158.

Vuillemin, P. (1912). *Beauveria*, noveau genre de Verticilliacies. *Bulletin de la Societe Botanique de France* **59**, 34-40.

Wang, Y. (1987). Mycology in ancient China. *The Mycologist* **1**, 59-61.

Wang, S.B., Miao, X.X., Zhao, W.G., Huang, B., Fan, M.Z., Li, Z.Z. & Huang, Y.P. (2005). Genetic diversity and population structure among strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-simple sequence repeats (ISSR). *Mycological Research* **109**, 1364-1372.

Watson, W. (1764). XLIV. An account of the insect called the vegetable fly. *Philosophical Transactions of the Royal Society of London* **53**, 271-274.

Weir, A. & Beakes, G. (1995). An introduction to the Laboulbeniales: a fascinating group of entomogenous fungi. *The Mycologist* **9**, 6-10.

White, T., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to methods and applications* (ed. M.A. Innis) pp. 315-322. Academic Press: San Diego

Willoughby, B., Glare, T., Kettlewell, F. & Nelson, T.L. (1998). *Beauveria bassiana* as a potential biocontrol agent against the clover root weevil *Sitona lepidus*. *Proceedings of the New Zealand Plant Protection Conference* **51**, 9-15.

Woods, S. & Grula, E. (1984). Utilizable surface nutrients on *Heliothis zea* available for growth of *Beauveria bassiana*. *Journal of Invertebrate Pathology* **43**, 259-269.

Yokoyama, E., Yamagishi, K. & Hara, A. (2004). Development of a PCR-based mating-type assay for Clavicipitaceae. FEMS Microbiology Letters **237**, 205-212.

Zare, R. & Gams, W. (2001). A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. *Nova Hedwigia* **73**, 1-50.

Zimmermann, G. (2007a). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology* **17**, 553-596.

Zimmermann, G. (2007b). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology* **17**, 879-920.

Zimmermann, G. (2008). The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces*

fumosoroseus): biology, ecology and use in biological control. *Biocontrol Science and Technology* **18**, 865-901.

APPENDIX: Authorities for generic and specific fungal names used in the text

All authorities below were obtained from Mycobank entries (Robert, Stegehuis & Stalpers 2005. *The MycoBank engine and related databases*. http://www.mycobank.org).

Acremonium Link 1809

Akanthomyces Lebert 1858

Aschersonia Endlicher 1842

Aschersonia duplex Berkeley 1855

Beauveria Vuillemin 1912

Beauveria alba (Limber) Saccas 1948

Beauveria amorpha (Höhnel) Samson & H.C. Evans1982

Beauveria bassiana (Balsamo-Crivelli) Vuillemin 1912

Beauveria brongniartii (Saccardo) Petch 1926

Beauveria caledonica Bissett & Widden 1988

Beauveria densa (Link) F. Picard 1914

Beauveria malawiensis S.A. Rehner & Aquino de Muro 2006

Beauveria tenella (Saccardo) Siemaszko 1954

Beauveria velata Samson & H.C. Evans 1982

Beauveria vermiconia de Hoog & V. Rao 1975

Botrytis P. Micheli ex Haller 1768

Botrytis bassiana Balsamo-Crivelli 1835

Botrytis paradoxa Balsamo-Crivelli 1835

Botrytis tenella (Saccardo) Delacroix 1891

Byssochlamys Westling 1909

Cephalosporium Corda 1839

Clavaria sobolifera Hill ex Watson 1763

Coelomomyces opifexi Pillai & J.M.B. Smith 1968,

Conoideocrella D. Johnson, G.H. Sung, Hywel-Jones & Spatafora 2009

Cordyceps Fries 1833

Cordyceps bassiana Z.Z. Li, C.R. Li, B. Huang & M.Z. Fan 2001

Cordyceps brongniartii Shimazu 1988

Cordyceps cardinalis G.H. Sung & Spatafora Sung, G.H. & Spatafora, J.W 2004,

Cordyceps cylindrica Petch 1937

Cordyceps memorabilis (Cesati) Cesati1861

Cordyceps militaris (Linnaeus) Link 1833

Cordyceps pseudomilitaris Hywel-Jones & Sivichai 1994,

Cordyceps robertsii (Hooker) Berkeley 1855

Cordyceps scarabaeicola Kobayasi 1976

Cordyceps sinclairii Berkeley 1855

Cordyceps sobolifera (Hill ex Watson) Berkeley & Broome 1875

Cordyceps staphylinidicola Kobayasi & Shimizu 1982

Cordyceps takaomontana Yakushiji & Kumazawa 1941

Engyodontium de Hoog 1978

Fusarium Link 1809

Gibellula Cavara 1894

Hirsutella Patouillard 1892

Hirsutella thompsonii F.E. Fisher 1950,

Hymenostilbe Petch 1931

Hypocrella Saccardo 1878

Isaria Persoon 1794

Isaria amoenerosea Hennings 1902

Isaria amorpha Höhnel 1909

Isaria cateniannulata (Z.Q. Liang) Samson & Hywel-Jones 2005

Isaria cateniobliqua (Z.Q. Liang) Samson & Hywel-Jones 2005

Isaria cicadae Miquel 1838

Isaria coleopterorum (Samson & H.C. Evans) Samson & Hywel-Jones 2005

Isaria farinosa (Holmskjold) Fries 1832

Isaria felina (DeCandolle) Fries 1832

Isaria fumosorosea Wize 1904

Isaria ghanensis (Samson & H.C. Evans) Samson & Hywel-Jones2 005

Isaria javanica (Friedrichs & Bally) Samson & Hywel-Jones 2005

Isaria orthopterorum Petch 1933

Isaria sinclairii (Berkeley) Lloyd 1923

Isaria sulphurea Fiedler 1859

Isaria tenuipes Peck 1879

Lecanicillium W. Gams & Zare 2000

Massospora.Peck 1879

Metarhizium Sorokin1879

Metarhizium anisopliae (Metschnikoff) Sorokin 1883

Moelleriella Bresadola 1896

Nomuraea Maublanc 1903

Nomuraea atypicola (Yasuda) Samson 1974

Nomuraea rileyi (Farlow) Samson 1974

Orbiocrella D. Johnson, G.H. Sung, Hywel-Jones & Spatafora 2009

Ophiocordyceps Petch 1931

Ophiocordyceps robertsii (Hooker) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora 2007

Ophiocordyceps sinensis (Berkeley) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora 2007

Paecilomyces Bainier 1907

Paecilomyces breviramosus Bissett1979

Paecilomyces cicadae (Miquel) Samson 1974

Paecilomyces farinosus (Holmskjold) A.H.S. Brown & G. Smith 1957

Paecilomyces lilacinus (Thom) Samson 1974

Paecilomyces ramosus Samson & H.C. Evans 1974

Paecilomyces rariramus Z.Q. Liang & B. Wang 2003

Paecilomyces tenuipes (Peck) Samson 1974

Paecilomyces variotii Bainier 1907

Paecilomyces xylariiformis (Lloyd) Samson 1974

Penicillium Link 1809

Pseudeurotium bakeri C. Booth 1961

Pseudeurotium J.F.H. Beyma 1937

Ramaria farinosa Holmskjold 1781

Regiocrella Chaverri & K.T. Hodge 2005,

Samuelsia P. Chaverri & K.T. Hodge 2008

Simplicillium lamellicola (F.E.V. Smith) Zare & W. Gams 2001

Simplicillium lanosoniveum (J.F.H. Beyma) Zare & W. Gams 2001

Sphaeria basili Taylor 1855 [a]

Sphaeria Haller 1768

Spicaria farinosa (Holmskjold) Vuillemin 1911

Spicaria Harting 1846

Sporotrichum Link 1809

Sporotrichum globuliferum Spegazzini 1880

Strongwellsea A. Batko & Weiser 1965,

Talaromyces C.R. Benjamin 1955,

Teberdinia Sogonov, W. Gams, Summerbell & Schroers 2005

Thermoascus Miehe 1907

Tolypocladium W. Gams 1971

Tolypocladium extinguens Samson & Soares 1984

Torrubia caespitosa Tulasne & C. Tulasne 1865

Torrubiella Boudier 1885

Torrubiella gonylepticida (A. Møller) Petch 1937[b]

Torrubiella pulvinata Mains 1949

Verticillium Nees1817

Verticillium griseum (Petch) W. Gams 1971

Verticillium lecanii (Zimmermann) Viégas 1939

[a] Date given incorrectly as 1844; [b] Date not given.

ACKNOWLEDGEMENTS

Many thanks to my senior supervisor Dr Tony Cole for his patience throughout this project.

Also thanks to Tony for giving me the initial opportunity to work in mycology.

I am extremely grateful to Dr Travis Glare and AgResearch for funding and the use of laboratory facilities.

Dr Peter Buchanan, Dr Peter Johnson, Dr Shaun Pennycook, Dr David Orlovich, Dr Jerry Cooper and others for organising the Landcare Fungal Forays.

Dr Nigel Hywel-Jones for his warm hospitality in Thailand and ongoing advice.

Matt Walters for his superb photography and help with digital imaging and printing.

David Tattle, Rennie Bishop and Dr Gerhard Lang for field assistance.

Tracey Nelson and Nicky Richards for their instruction in molecular techniques.

Dr Michael Brownbridge and Dr Steve Reay for help with bioassays, and many useful discussions.

Dr Cor Vink for his advice on phylogenetics.

Dr Alexis Guerin and Manfred Ingerfeld for French and German translation, respectively.

Dr Ermin Schadich for his kind assistance on various levels.

I would also like to thank my family for their wonderful support and patience.